THESIS

MEASURING THE EPOXY CONTENT OF AEROSOLS USING ION CHROMATOGRAPHY

Submitted by

Jay Andrew Vietas

Department of Environmental Health

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2000

REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	17.Jul.00	THESIS

4. TITLE AND SUBTITLE
MEASURING THE EPOXY CONTENT OF AEROSOLS USING ION CHROMATOGRAPHY

5. FUNDING NUMBERS

6. AUTHOR(S)
CAPT VIETAS JAY A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
COLORADO STATE UNIVERSITY

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
THE DEPARTMENT OF THE AIR FORCE
AFIT/CIA, BLDG 125
2950 P STREET
WPAFB OH 45433

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
FY 00-257

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Unlimited distribution
In Accordance With AFI 35-205/AFIT Sup 1

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

14. SUBJECT TERMS	15. NUMBER OF PAGES 103
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

COLORADO STATE UNIVERSITY

May 12, 2000

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUT SUPERVISION BY JAY ANDREW VIETAS ENTITLED *MEASURING THE EPOXY CONTENT OF AEROSOLS USING ION CHROMATOGRAPHY* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

Dr. Philip Bigelow

Dr. John Tessari

Dr. Jim Loftis

Department Head: Dr. John Reif

ABSTRACT OF THESIS

MEASURING THE EPOXY CONTENT OF AEROSOLS USING ION CHROMATOGRAPHY

This study was designed to determine if ion chromatography was an adequate analytical tool for measuring the concentration of reactive epoxy resins in ambient air as well as to determine standard stability of the method for use in field industrial hygiene operations. Stoichiometric reaction of bromide generated *in situ* with spiked standards of a 89.4 percent pure and 93.8 percent pure model compound, diglycidyl ether of bisphenol A (DGBA), were analyzed using ion chromatography to determine recovery rates of the model compound. Recovery rates for five different loading levels were compared to guidelines set forth by the National Institute for Occupational Health and Safety (NIOSH) in order to assess the outcome of this method. Standards were analyzed six times over a 30 day period in order to determine standard stability.

Mean recovery rates for both of the DGBA products met the NIOSH guidelines for the highest two loading levels, but not the lower three loading levels. Coefficients of variation were also high for the lower three loading levels, much greater than 10, and were generally much less than 10 for the two highest loading levels. The results suggested carryover, contamination from one standard to another, as the cause of the poor recovery rates as well as the large variations in the data. Eliminating the carryover should significantly improve the results.

Standard stability was determined to be acceptable for field industrial hygiene operations. Significant differences were determined for the highest two loading levels between the initial reaction time and times of interest. Linear models were developed to explain the reduction in standard stability and the maximum time that the standards still met guidelines set forth by NIOSH was determined to be 20 days.

Jay Vietas
Department of Environmental Health
Colorado State University
Fort Collins, Colorado 80523
Summer 2000

ACKNOWLEDGMENTS

I would like to thank the members of my committee, Dr. Philip Bigelow, Dr. John Tessari, and Dr. Jim Loftis for their guidance and support throughout this endeavor. I would also like to thank the National Wildlife Research Center and the United States Department of Agriculture for allowing me to use their facilities and instrumentation throughout this project. In addition, I would like to thank Major Ellen England and Lt Col Gary Carlton, United States Air Force, for their encouragement and financial suppport for this project. Finally, I would like to extend a special thank you to Mr. David Goldade for contributing his time and energy to help me effectively use the instrumentation and conduct data analysis for this project.

DEDICATION

I would like to dedicate this work to my wife Jennifer who gave unconditional love, guidance, and support throughout this project. Without you, none of this would have been possible. I would also like to extend my appreciation to my two children, Cassandra and Christopher, who inspire me every day by the desire to live life to its fullest. Finally, I would like to thank my extended family for their lifelong support, making all of this possible.

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CHAPTER I

INTRODUCTION

Epoxy resins are found virtually everywhere. They are used in paints, adhesives, caulking compounds, sealants and are even found in electronic equipment. Impressive characteristics of low shrinkage, high adhesive strengths, outstanding mechanical and electrical properties, and their resistance to chemicals have made epoxy resins a popular choice for employment (Lee, 1967). Over the years, use of epoxy resins has continued to increase. Manufacturing, consumer use, and exports are all on the rise (Society of the Plastics Industry Committee on Resin Statistics, 1998). Despite the outstanding properties of epoxy resin systems, worker exposure to epoxy resin systems can result in a wide range of adverse health effects.

Contact allergic dermatitis has been found to be one of the most common adverse effects of exposure to epoxy resins. In fact, approximately seven percent of all occupational skin disease cases is caused by epoxy resins, with the monomer form of digylcidyl ether of bisphenol A (DGBA) as the likely cause (Jolanki et al., 1990). Unfortunately, allergic contact dermatitis is not a disease that workers can simply ignore. It is an allergic reaction which, in many cases, can result in the worker not being able to perform his or her job ever again.

Although researchers have been aware of the sensitizing potential of epoxy resins for many years, there is limited information on the cause and effect relationship between

exposure and adverse health effects. In fact, no studies have been performed relating inhalation exposure to epoxy resins and adverse health effects. One reason is that DGBA does not have a measurable vapor pressure and consequently, inhalation studies are difficult. This lack of an approved analytical method of measuring worker exposures to DGBA has prevented any direct link between worker exposures and any future health problems.

DGBA, like other monomers, reacts in the presence of a curing agent to form a polymer. Since typical industrial hygiene methods rely on adsorption or gravimetric techniques, measurement of the original chemical, DGBA, is difficult in the workplace. Effective measurement techniques must stop the reaction, stabilize DGBA, and allow for measurement once transported to the laboratory.

In 1987, Dr. Robert Herrick, Harvard School of Public Health, developed a method that would accomplish all three of these tasks. It involved using dimethylformamide as a solvent which stopped the polmerization process, stabilized the remaining DGBA, and allowed for measurement of DGBA once it was transported to the laboratory. The process required the addition of bromide ion to the standard, which was then later measured to determine the amount of bromide ion consumed (Herrick & Smith, 1987). Unfortunately, the measurement of bromide ion required the use of normal pulse polarography, an uncommon analytical instrument. Even if this method were to be approved for use, it is unlikely that laboratories would purchase this instrument just for one analytical method.

About the same time that Dr. Herrick was conducting his experiments, ion chromatography became a practical and popular analytical instrument to measure both

anions and cations. In fact, since the mid-1980's, many analytical methods have been developed using ion chromatography to measure chemicals in both the workplace and the environment (National Institute for Occupational Safety and Health, 1994). With ion chromatography's versatility, many labs already have the equipment in place. Ion chromatography is fast, sensitive, and reliable (Weiss, 1995).

This thesis intends to extend the method developed by Dr. Herrick to using ion chromatography as an analytical tool for determining concentrations of DGBA. The work is necessary for furthering the science of understanding the relationship between worker exposure and adverse health effects. Additionally, the work begins the pursuit for an approved analytical method for determining epoxy resin exposure.

Ultimately, the goal is for workers, toxicologists and industrial hygienists to know more about epoxy resin exposure. They will know when it is safe to work without personal protective equipment without the fear of lifelong sensitization. They will know which studies to perform in order to understand the mechanism of toxicity. Finally, they will know how to measure for epoxy resins in order to determine worker exposure.

CHAPTER II

LITERATURE REVIEW

According to Lee, "the term epoxy refers to a group consisting of an oxygen atom bonded with two carbon atoms already united in some other way" (Lee, 1967). The simplest epoxy is a three membered ring such as ethylene oxide:

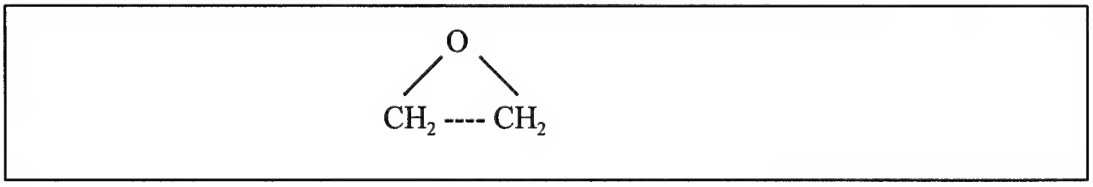

Figure 1 -- Molecular Structure of an Epoxy Group

An epoxy can be defined as a molecule containing at least one epoxy group. Typically, epoxy resins are chemically prepared by reacting epichlohydrin with bisphenol A to form digylcidyl ether of bisphenol A (Lee, 1967). Also, by changing the precursor ratio and/or the process, "a series of commercial products is available, categorized by the number of bisphenol A repeating units, known as oligomers, and by molecular weight"(Holmes, Pearce, & Simpson, 1993). The smallest possible epoxy resin molecule, when n=0, is known as the monomer. The monomer structure for diglycidyl ether of bisphenol A is found below:

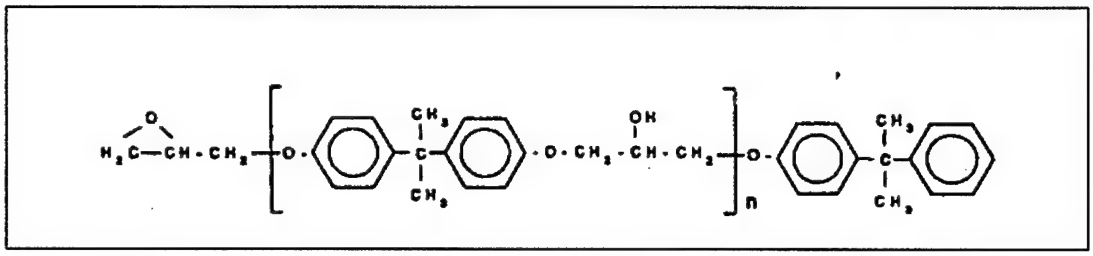

Figure 2 -- Molecular Structure of Digylcidyl Ether of Bisphenol A (CAS # 1675-54-3)

Credit for the synthesis of epoxy resins using epicholorhydrin is shared by Dr. Pierre Castan of Switzerland and Dr. S.O. Greenlee of the United States in 1927. Their work resulted in an explosion of research into epoxy resins over the next 20 years. During that period it was found that epoxy resins formed low viscosity products that were easy to cure, had low shrinkage, high adhesive strengths, high mechanical properties, were excellent electrical insulators and very chemically resistant. As a result, many industrial activities began to use epoxy resins for adhesives, caulking compounds, sealants, impregnation resins for electronic equipment, as well as solution coatings (paints) for all types of surfaces (Lee, 1967).

Despite the outstanding properties of epoxy resin systems, worker exposure to epoxy resin systems can result in a wide range of adverse health effects. The most common effect is occupational allergic contact dermatitis (Jolanki et al., 1990). Jolanki states that out of 3731 patients investigated between 1974 and 1990 for occupational allergic contact dermatitis, 142, or 7.7 percent, had an occupational skin disease caused by epoxy compounds. Jolanki also found that sensitizing potency is a function of both chemical composition and molecular weight. Diglycidyl ether of bisphenol A (DGBA), in monomer form, has been found to be the main cause for this delayed hypersensitivity

(Le Coz et al., 1999). The differences in the monomer form and the higher molecular weight forms may be explained by the differences in size between the two molecules. If the toxicity of epoxy resins is due to the reactive epoxy group on the end of epoxy resin molecule, then when a higher molecular weight epoxy or a lower molecular weight epoxy comes in contact with the worker, then each would interact to cause the same health effects. However, on a mass basis, the same amount of monomer and higher molecular weight oligomer will have differing amounts of reactive sites, with the monomer having more reactive sites per mass.

Chronic exposure to epoxy resins have been known to cause burning of the eyes, blistering of the hands and face, coughing, irritation of the upper respiratory tract, and increased nasal secretions. Documentation of the skin-sensitizing potential of epoxy resins dates back to the early 1950's (Thorgeirsson & Fregert, 1977). This information was then used by the National Institute of Occupational Safety and Health (NIOSH) which in 1976 published a pamphlet titled, "Epoxy Wise is Health Wise", warning workers of the potential for sensitization, eye injury, dizziness, explosion, and even death. NIOSH recommended the use of gloves, eye protection, as well as respiratory protection to ensure safe use of epoxy resin systems (National Institute for Occupational Safety and Health, 1976). Unlike most other widely used chemicals, safe exposure levels were not determined for the worker.

Since that time, many studies have been performed relating the adverse effects of epoxy resin exposure to direct contact with the chemical. In March 1974, NIOSH conducted a health hazard evaluation at Head Ski Company in Boulder, Colorado. Approximately 300 workers, operating in three 8 hour shifts, would come in contact with

many of the chemicals in the plant to include epoxy resin Dow-330. Dow-330 is a liquid epoxy resin which contains a significant portion of DGBA for a Dow product. It is very similar to Dow-332 which has the highest level of DGBA. The actual amount of DGBA is not divulged, but as expressed in "Epoxide Equivalent Weight". Pure DGBA would have an epoxide equivalent weight of 170 (170 grams per mole). Dow 332 has an epoxide equivalent weight of 172-176 and Dow 330 has an epoxide equivalent weight of 176-185. The increase epoxide equivalent weight is due to the higher molecular weight epoxies (The Dow Chemical Company, 1998). Of 20 employees (17 women and 3 men) at the Head Ski Company with recurrent dermatitis, twelve of these workers were patch tested for epoxy resin-Dow 330 and six workers tested positive. On average, seventeen months was the service time for those who tested positive and seven months was the service time for those who did not test positive for epoxy resin (National Institute for Occupational Safety and Health, 1974). More recently, in a separate study of 22 ski factory workers, six developed allergic contact dermatitis from epoxy resin compounds, and four had irritant contact dermatitis (Jolanki et al., 1996). Another study of 62 workers in Iraq who were exposed to paint and 34 workers who were not exposed to paint were patch tested for a variety of chemicals to include epoxy resin. 41.9 percent of the paint workers were positive for at least one allergen with 20.9 percent positive for epoxy resins. Only one worker not exposed to paint was positive for any allergen; and in this case it was to epoxy resin (Omer & al-Tawil, 1994).

Many researchers discount airborne contact with epoxy resins mainly because the vapor pressure of DGBA is so low. In fact, Nolan *et al.* reported difficulties in generating an atmosphere to conduct inhalation studies in rats, as explained by an absent vapor

pressure for DGBA (Nolan, 1981). Consequently, inhalation exposure studies, either chronic or subchronic are not found in the literature. However, epoxy resins are typically applied as a surface coating by creating an aerosol of the product and thereby suspending the chemical in the air.

> In the painting industry, occupational exposure to epoxy resins is often by spray application...spray painting allows inhalation of, and skin contact on all parts of the body with, both volatile and non-volatile components. (Holmes et al., 1993)

This method of application would result in airborne contact with epoxy resin constituents. There is some literature that supports a relationship between adverse health effects and airborne contact with the epoxy resins. Two cases, a 17 year old male who applied epoxy resin to lighters, and a 63 year old female who painted key rings, developed lesions on the face, eyelids, and nose areas. The author felt strongly that airborne contact dermatitis was the cause of the sensitivity (Ortiz-Frutos, Borrego, Romero, & Iglesias, 1993). Additionally, one study of shipyard workers found a significant relation between percent decrement in forced expiratory volume and hours of exposure to epoxy paints. Pre and post-shift spirometry measuring forced vital capacity and forced expiratory flow for each painter was performed. Participants were excluded if (1) they were exposed to epoxy paints the preceding day, (2) if exposures were not uniform (i.e. were supervisors) or (3) if they had severe chronic obstructive pulmonary disease. Painters exposed to epoxy paints for more than 15 minutes were classified as "exposed" and all others were classified as "non-exposed". The mean change in forced expiratory volume for "exposed" painters was –3.4 percent, while "non-exposed" workers had a change of –1.4 percent. Furthermore, a significant linear relationship was found between hours of

exposure and percent decrease of forced expiratory volume (Rempel, Jones, Atterbury, & Balmes, 1991). In June 1981, NIOSH conducted a health hazard evaluation at a General Dynamics Shipyard in Quincy Massachusetts which employed 2400 workers. One process involved performing "hot work" inside a submarine repair facility, which was the application of surface coatings in an elevated temperature environment. Both male and female workers complained of irritation, chest tightness, chest pain, and nausea, although specific numbers were not reported (NIOSH, 1981). In a separate review of 40 cases diagnosed with occupational skin dermatitis induced by current occupational exposure to DGBA, 21 patients (or 53 percent) had skin symptoms on their face, suggesting airborne contact dermatitis (Jolanki et al., 1990). Again, despite the epidemiological trends, neither aerosol or vapor inhalation studies are found in the literature to support or refute specific cause effect relationships.

To explain the cause of the sensitization and irritation, toxicokinetic models have been proposed for glycidyl ethers which are similar to those of other epoxide compounds. One metabolic pathway involves the enzyme epoxide hydrolase which in the presence of water to creates a diol. Another metabolic pathway involves glutathione-s-epoxide transferase which adds a hydroxyl group and a sulfhydryl group. The third proposed model is a non-enzymatic mechanism by which the epoxide group, which has a very short half life, covalently bonds with proteins, RNA, and DNA, suggesting that DGBA is genotoxic (Bos, 1992). The last model, non-enzymatic mechanism by which the epoxide group and covalent bonding with proteins, RNA, and DNA, is best accepted as the cause of allergic contact dermatitis (Herrick & Smith, 1987). Therefore, to prevent adverse

health effects, measures should be taken to prevent this interaction, and worker exposure models should focus on the reactive nature of the epoxide groups.

Epoxy resin systems were once thought to be related to an increase cancer risk for both males and females, however more recent studies have not confirmed this relationship. Zakova *et al.* conducted study in which groups of 50 CF1 mice of each sex were treated with acetone (solvent), as well as with 1 % and 10 % DGBA solution in acetone. Additionally, treated groups of 50 mice of each sex with acetone and with a 2 percent solution of B-propiolactone. Treatment included application of 0.2 ml on a 1 cm^2 area of dorsal skin twice a week for two years. Similar lesions (non-malignant tumors) were seen with the acetone alone and with the solutions of DGBA. However, the control chemical, B-propiolactone, induced a high incidence of lesions (both malignant and non-malignant). This study showed that DGBA did not cause skin carcinogenicity or any hematologic or clinical chemistry changes from skin application of DGBA in CF1 mice (Zakova, Zak, Froehlich, & Hess, 1985). No cases of cancer to humans as a result of exposure to DGBA have been documented in the literature.

It has been estimated that over 3000 aircraft corrosion control workers in the United States Air Force are routinely exposed to epoxy resins (England, 1999). In 1997, the United States used over 506 million pounds of epoxy resins, 53 percent of which are used for protective coatings (Society of the Plastics Industry Committee on Resin Statistics, 1998). Since 1993, epoxy resin consumption gradually increased over 22 percent, or an average of 5.2 percent per year (Society of the Plastics Industry Committee on Resin Statistics, 1998). Imports of epoxide resins have increased from 59 million dollars worth of product in 1992 to over 140 million dollars in 1997 (International Trade

Commission, 1999). In 1993, 121 million pounds of epoxy resins were exported compared to 144 million pounds in 1997; an increase of 19 percent (Society of the Plastics Industry Committee on Resin Statistics, 1998). Although these dramatic increases in production may not necessarily mean increased numbers of workers are exposed to epoxy resins, it certainly is more likely that either the risk of exposure to workers is increasing or that the number of workers exposed is increasing. If this is the case, then the need for relating health effects from epoxy resin exposure with the amount of epoxy resin that a worker is exposed to becomes paramount. For this reason, an organization like the United States Air Force has decided to protect literally thousands of workers with supplied air respiratory protection and tyvek suits when exposed to epoxy resins. This is a costly proposition in terms of equipment costs, reduced worker productivity, as well as increased medical costs for fit testing and physical exams. Unfortunately, all this expense does not help in the understanding of how chemical sensitization occurs or why it occurs. Multiply these costs by the amount of epoxy resins being used in the United States and worldwide and control of these epoxy resins is important to the health of a significant portion of the human population.

Typical industrial hygiene methods of determining worker exposure are based upon mass or count concentration of chemicals collected on a filter. Although the health effects of exposure to epoxy resins are not known with certainty, "the effects are probably the result of the epoxide functional group reacting with proteins and other nucleophilic substances" (Herrick, Smith, & Ellenbecker, 1987). Additionally, a basic industrial hygiene principle is to look at the source, path, receiver model of exposure and control of this exposure focuses on disrupting one or more parts of the model. In this case, it is

important to distinguish between the amount of chemical that is aerosolized and the amount of chemical that reacts with the worker. Therefore, to directly measure the health effects of the epoxy resin, the amount of reactive epoxy groups that can potentially react with the worker must be measured in order to adequately associate chemical exposures with health effects. Unfortunately, the amount of reactive epoxies present is not a constant. This is because the amount of reactive epoxies will depend upon how much of the product has reacted with each other, and other substances, before reaching the worker. Therefore, if the reactive epoxy does not reach the worker, then the worker is not exposed. Factors that affect the amount of reactive epoxies present are the same factors that affect the curing time of the epoxy resin. Temperature is probably the most important factor, but humidity would also affect the amount of free epoxy. Therefore the method for determining the health effects of epoxy resins must include the following characteristics. It must stop the reaction between the epoxy and the curing agent and it must also be able to measure the amount of unreacted epoxide content of the aerosol as it existed at the point of human exposure. This approach is not seen elsewhere in industrial hygiene methods, but from a toxicological point of view it does make the most sense.

In order to assess worker exposure, Dr. Robert Herrick, Harvard School of Public Health, used the chemical principles from ASTM Method 1652-90 used by industry to determine the amount of reactive epoxy resins in paints. This method relies on a change of pH which can be interfered with in an occupational setting due to the use of amine curing agents which themselves can change the pH. The method also relies on direct titration which is not very sensitive and is "limited by the speed of hydrobromination reaction, which is very slow at low epoxide concentrations resulting in indistinct

endpoints." (American Society for Testing and Materials, 1983). This method has been documented as having an ability of detecting 170 mg of epoxy resin or 1 milliequivalent (Selig & Crossman, 1971). Selig and Crossman used the principle of hydrobromination with a 2 molar excess of bromide generated *in situ* using tetraethylammonium bromide (TEAB) resulting in the ability to detect standards containing as little as 5 mg of epoxy resin or 0.03 milliequivalents. Dr. Herrick's work expanded on this procedure. His method involved collecting standards of pure diglycidyl ether of bisphenol A (DGBA), a model epoxy compound, and placing it into a solution of dimethyl formamide (DMF). DMF was chosen after testing 18 compounds with good solvent properties for DGBA based upon the fact that it solubilized the DGBA, did not react with the epoxide, and it inhibited the reaction between the epoxy and the curing agent. The procedure then required the addition of TEAB to the solution of DMF. When combined, the stoichiometric hydrobromination of the epoxide group by the bromide, generated *in situ* from the TEAB, occurs. This reaction is favorable in an acidic environment. The reaction is as follows:

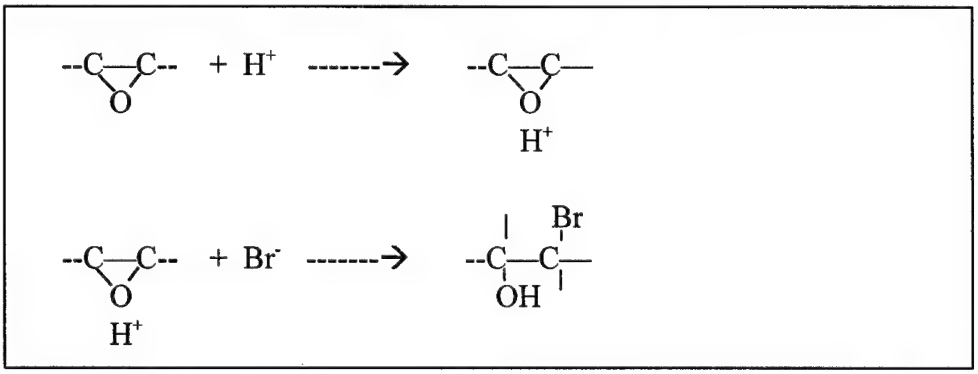

Figure 3 -- Hydrobromination Reaction of the Epoxide Group.

DGBA was chosen as a model epoxy compound for two reasons. One, the epoxy resins used in commercial surface coatings are usually mixtures containing DGBA and

higher molecular weight homologues of DGBA. Two, the analytical method determines the total reactive epoxy content, regardless of the identity of the specific molecules. Therefore, by using DGBA in a laboratory environment, theoretical comparisons to determine analytical accuracy can be accomplished.

Once the epoxy was collected in the sampling media and reacted with a known amount of TEAB, the amount of bromide present was determined using normal pulse polarography (NPP) as the analytical instrument. The amount of reactive epoxy resin collected was determined by the amount of bromine consumed during the reaction (Herrick et al., 1987). Dr. Herrick determined that by using this method, standards containing as little as 0.17 milligrams, or 1 microequivalent of DGBA could be detected. Also, using a guideline set forth in the NIOSH Manual of Analytical Methods, he determined that all standards met the requirement of 75 percent recovery rate 95 percent of the time (National Institute for Occupational Safety and Health, 1994). Additionally, he found that the reaction between the epoxide and the bromide was approximately 90 percent complete at 4 hours, and that the method could be applied to examination of generated atmospheres of DGBA to determine airborne concentrations (Herrick et al., 1987). However, since 1987 when this work was accomplished, the National Institute for Occupational Safety and Health (NIOSH) has not adopted this procedure as an analytical method for determining epoxy resin exposure. In fact, additional work on this method has not been published.

Although NPP is an adequate analytical tool for determining the reactive epoxy resin content, currently there are not any proposed or existing NIOSH or Environmental Protection Agency standards which require the use of NPP. Therefore, most analytical

laboratories that are accredited to analyze NIOSH or EPA methods cannot perform NPP. Instruments that are common to these laboratories are not able to perform the anion analysis necessary for determining bromide concentrations.

> Whereas in the field of cation analysis both fast and sensitive analytical methods are available (AAS, ICP, polarography, and others), the lack of corresponding, highly sensitive methods for anion analysis is noteworthy. The conventional methods such as titration, photometry, gravimetry, turbidity, and colorimetry are all labor intensive, time consuming and occasionally troublesome. (Weiss, 1995).

For these reasons, an alternate analytical tool, ion chromatography, was chosen for this analysis.

Some of the advantages of ion chromatography include speed, sensitivity, and the stability of the separator columns. Ion chromatography is fast. According to Weiss, the average analytical time is ten minutes and in most cases requires only three minutes. Ion chromatography is also sensitive enough to determine concentrations down to the part per billion level. The packing for the ion chromatograph is also very stable due to the use of resin materials which allow for pH stability allowing for the use of strong acids and bases as eluents. Currently there are eleven methods approved by NIOSH that require the use of ion chromatography as an analytical method (National Institute for Occupational Safety and Health, 1994). Additionally, EPA Method 300.0 requires ion chromatography for determining chloride, nitrate, phosphate and sulfate in discharge waters using EPA Method 300.0 (Karmarkar, 1996). There are also additional uses for ion chromatography which include determining chlorite and chlorate levels in drinking water (Dietrich, 1992).

One of the problems with the method suggested by Dr. Herrick is that it requires taking area air samples as opposed to personal air samples. This would mean that air

sampling results would not be representative of individual worker exposure, but instead would be representative of the amount of reactive epoxy resins in a specific area. Since workers perform tasks that require them to move around constantly during operations as well as frequent starts and stops of operation, area samples would not be as representative of worker exposure as personal air samples. The reason for the recommended use of area versus personal sampling is due to the use of dimethylformamide (DMF) in the midget impingers when collecting samples.

DMF is an excellent industrial solvent due to its polar properties and slow evaporation rate and has been labeled by some as the "universal organic solvent" (Budavari & Merck & Co., 1989). Although DMF does not evaporate readily, it does have a vapor pressure of 3.7 mmHg which provides a significant pathway for inhalation exposure. Additionally, DMF is readily absorbed by the skin through both contact exposures as well as during exposures to gaseous levels of DMF (National Library of Medicine, 1999). As a water soluble compound, once DMF enters the body it is readily transported through the blood. DMF is a polar compound and therefore it is less likely to be diffuse into tissues and is excreted in the urine by the kidneys. The estimated half life of DMF was one to two hours in monkeys suggesting that the fate of the majority of DMF is determined by biotransformation (Hundley, McCooey, Lieder, Hurtt, & Kennedy, 1993). The primary toxic effect of DMF occurs locally at the location of biotransformation; the liver. Hepatocyte death occurs through necrosis, which is the swelling, leaking, nuclear disintegration and influx of inflammatory cells (Casarett, Klaassen, Amdur, & Doull, 1996). Primarily chronic exposures to humans cause this type of liver damage, however, high acute exposures can cause liver damage which has

been found to be reversible (National Library of Medicine, 1999). The primary measure of the toxic effect is through the monitoring of transaminases, aminotransferase (ALT) and aspartate aminotransferase (AST). Increased damage to the liver causes the production of these transaminases. Elevated transaminase levels were seen in 35 of 46 production workers tested that were chronically exposed to DMF (NIOSH, 1990a). DMF is a skin and eye irritant (Lewis, 1993), which can result in contact dermatitis, including itching and desquamation of the skin. More importantly, DMF is easily absorbed by the skin and contributes to body burden along with other routes of exposure. DMF has also been shown to cause kidney damage (National Library of Medicine, 1999) and hypertension in animals (Imbriani et al., 1986).

Many studies have been conducted to determine the carcinogenicity of DMF without resolve. As a result, the International Agency for Research on Cancer (IARC) has classified the link between DMF and cancer as "inadequate" but on the basis of published literature has it classified as a IARC Group 2B, "possibly carcinogenic to humans" (NIOSH, 1990a).

Numerous studies have been performed on animals using DMF. Additionally, due to the large volume of use, many case studies of human exposure to DMF have been documented as well. To date, no human fatalities have been documented by exposure to DMF (National Library of Medicine, 1999). This is consistent with high LD_{50} oral doses; greater than 1500 milligrams per kilogram, for rats and mice (National Library of Medicine, 1999). Additionally, five (5) six hour doses of 2500 part per million DMF were needed to kill rats (ACGIH, 1991). Despite this, elevated ALT levels were found in workers exposed to 25 to 60 part per million, suggesting liver damage for chronic DMF

exposures (Wang et al., 1991). This was also demonstrated in a matched pair study of 100 DMF workers. The five year study showed increased gamma-glutamyl transpeptidase levels when exposed to continuous occupational exposures of greater than 7.5 part per million (Cirla, Pisati, Invernizzi, & Torricelli, 1984). Studies of the dermal effect of DMF show exposures of 3600 milligram per kilogram before a lethal response (National Library of Medicine, 1999). One accidental human dermal exposure of 20 percent of the body resulted in redness, dermal irritation and vomiting with recovery in seven days (Potter, 1973). None of these studies show acute health effects from exposure to DMF.

Despite the fact that there is evidence that chronic DMF exposure can result in adverse effects, there is little evidence that short term exposure to DMF causes adverse effects. The risk involved from inhalation exposure to DMF compared to other impinger methods approved and outlined by the NIOSH Manual of Analytical Methods is similar (National Institute for Occupational Safety and Health, 1994). A comparison of the ratios of the vapor pressure (VP) in Pascals (Pa), to the threshold limit value (TLV),as listed by ACGIH in parts per million (ppm), or vapor hazard index (VHI) shows similar inhalation risks for the proposed method and other approved methods. The method of calculating the vapor hazard index is accomplished using the following equation:

$$VHI = \frac{VP}{TLV}$$

Equation 1 -- Calculation of the Vapor Hazard Index

The sampling method for isocyanates and toluene diisocyanate requires the use of toluene, vapor pressure of 2900 Pa, and a TLV 50 ppm with a resulting VHI of 58. The method for sampling tetranitromethane requires ethyl acetate, vapor pressure of 10 kPa, and a TLV of 400 ppm with a resulting VHI of 25. The proposed method for sampling DGBA requires DMF, vapor pressure equal to 492 Pa, a TLV of 10 ppm with a calculated VHI of 49. Since the inhalation risk is essentially the same for DMF as for the other impinger methods, the proposed method should be approved for individual sampling use in order to accurately determine individual worker exposure (National Institute for Occupational Health and Safety, 1999).

Adverse health effects from epoxy resin exposure have been conclusively demonstrated. Unfortunately, with the exception of animal studies, the relationship between the health effect and the environmental exposure is not well characterized due to the lack of an analytical method for determining the amount of reactive epoxy resin exposure for a worker. A simple method of using ion chromatography to determine the amount of bromide consumed during the reaction of the reactive epoxy resin and bromide can be used to quickly assess worker exposure and begin the process of relating adverse health effects to exposure levels.

CHAPTER III

PURPOSE AND SCOPE

If there are many workers exposed to epoxy resin systems, and a significant portion of those workers become sensitized from exposure, why has so little work been done to determine worker exposure? Simply put, the work is considered to be too complex, and the analytical tool, normal pulse polarography, is not readily available in even large industrial hygiene analytical operations. Consequently, worker exposures are not developed, which gives the National Institute of Occupational Safety and Health (NIOSH) little reason to set a worker exposure standard. As a result, the relationship between the health effects and exposure are not documented and consequently, little research interest is generated.

The purpose of this work is to demonstrate that a widely available analytical tool, ion chromatography, can be used to determine worker exposure to epoxy resins. Specifically, the work will show that ion chromatography can be used to repeatably and reliably determine concentrations of diglycidyl ether of bisphenol A (DGBA) in both pure form and in a relatively unpure form. Standard stability will also be investigated to determine how long standards remain viable and to develop a model that will explain the relationship between time and standard viability.

The first part of this project was performed in order to show that ion chromatography, as an analytical tool, is at least as effective as normal pulse

polarography, for determining the amount of DGBA present in a standard. This was accomplished by determining the recovery rates of DGBA at five different loading levels. Four standards at each of the loading levels were generated and analyzed using ion chromatography. Mean recovery rates, under standardized conditions, were determined for each of the different loading levels in order to assess the concentration at which DGBA can be detected. These values were compared with previous experiments conducted using normal pulse polarography in order to assess the differences between the two analytical tools. Additionally, coefficients of variation were calculated in order to determine the repeatability of the instrument for comparison with normal pulse polarography.

The second portion of this project determined the relationship between recovery rates and time. All of the standards analyzed in part one of this project were reanalyzed at multiple time periods to determine when the standards are no longer viable. This was accomplished in order to determine if this method was applicable for industrial hygiene sampling in field operations.

Although the results from this experiment were not directly applicable to field analysis, they provide insight as to the viability of this method. Furthermore, this study was not able to demonstrate whether the applied reaction procedures are the only or the best procedures to be followed when using an ion chromatograph. Additionally, the study will not be able to determine if these procedures are applicable to all mixtures of DGBA since only two concentrations of DGBA were investigated. Finally, this study had inherent limitations common to any laboratory experiment; controlled temperature, pressure, light, and humidity.

CHAPTER IV

METHODS AND MATERIALS

The purpose of this project was to demonstrate that a widely available analytical tool, ion chromatography, can be used to repeatably and reliably determine concentrations of diglycidyl ether of bisphenol A (DGBA) in both pure form and in a relatively unpure form. Furthermore, the shelf life of the standard was investigated to determine how long standards remain viable and to develop a model that will explain the relationship between shelf life and viability.

The project involved determining the recovery rates of DGBA at five different concentrations. Four standards at each of the five loading levels were generated and analyzed using ion chromatography. All of the standards were analyzed again at different time periods after the initial reaction procedure to determine standard viability.

This study was conducted at the National Wildlife Research Center (NWRC) in Fort Collins, Colorado. A part of the United States Department of Agriculture, the NWRC is a top-notch facility designed to adequately and safely conduct research experiments using analytical chemistry techniques. One of two ion chromatographs on hand, purchased from Dionex in the middle of the 1980's, was used to perform this study. In order to reach the goals of the project, the research was conducted in a series of five steps or procedures. The procedures included:

1. Setup of the ion chromatograph to determine bromine levels.
2. Determining the reagents and quantities for reaction.
3. Executing the reaction procedures.
4. Obtaining and determining different purity levels of digylcidyl ether of bisphenol A using gas chromatography.
5. Performing data analysis.

1.0 Setting up the Ion Chromatograph

1.1. Chemicals, Reagents, Solutions:

1.1.1. Sodium bromide (Sigma Chemical, St. Louis, MO) 99.5 percent purity. Dried for four hours at 110 °C and stored in a dessicator prior to use

1.1.2. 0.2 micron deionized water

1.1.3. Sulfuric acid concentrated (Acros Organics, Fair Lawn, NJ), 95-97 percent purity

1.1.4. Sodium bicarbonate (Sigma Chemical, St. Louis, MO); dried for four hours at 110 °C and stored in a dessicator prior to use

1.1.5. Sodium carbonate (Sigma Chemical, St. Louis, MO); dried for four hours at 110 °C and stored in a dessicator prior to use

1.1.6. Glacial acetic acid (Acros Organics, Fair Lawn, NJ), 99.8 percent purity

1.1.7. Perchloroacetic acid (Sigma-Aldrich Chemical, Steinheim, Germany) 70 percent

1.1.8. Tetraethylammonium bromide (Acros Organics, Fair Lawn, NJ) 99.5 percent purity. Dried for four hours at 110 °C and stored in a dessicator prior to use

1.1.9. N,N-dimethylformamide (Acros Organics, Fair Lawn, NJ) 99.5 percent purity

1.1.10. Diglycidyl ether of bisphenol A (TCI America, Portland Oregon) 88.4 percent purity

1.1.11. Methyl ethyl ketone (Fisher Scientific, Fair Lawn, NJ) 99.8 percent purity

1.1.12. Toluene (Baxter Healthcare Corp., Muskegon, MI) 99.99 percent purity

1.2. Equipment/Apparatus

1.2.1. Fisher Isotemp Oven Forced Draft, SN # 1671

1.2.2. Dessicator

1.2.3. Analytical balance Mettler PM6100 balance, SN #1113081078

1.2.4. GlasCos Touch Vortex, Terre Haute, IN, SN #271091

1.2.5. Branson 5200 Sonicator, SN#B0792514013

1.3. Standard preparation:

1.3.1. Concentrated Standard: Using an analytical balance, 1.000 g of previously dried 99.5 percent pure sodium bromide was placed into a 100 mL volumetric flask. The sodium bromide was dissolved and diluted in deionized water to volume and mixed well. The concentration of the resulting solution was 9950 mg/L.

1.3.2. 0.995 mg/L: 0.1 mL was dissolved into a 1000 mL volumetric flask by diluting with deionized water. The solution was then vortexed to ensure good mixing.

1.3.3. 9.95 mg/L: 1.0 mL was dissolved into a 1000 mL volumetric flask by diluting with deionized water. The solution was then vortexed to ensure good mixing.

1.3.4. 99.5 mg/L: 1.0 mL was dissolved into a 100 mL volumetric flask by diluting with deionized water. The solution was then vortexed to ensure good mixing.

2.0 Determining Reagents and Reaction Procedures:

2.1.1. Based upon guidance provided by Mr. Mike Dammon, from Southwest Research Institute in San Antonio, Texas, the initial concentration of the eluent generated was a mixture of 2.8 mM sodium carbonate and 2.2 mM sodium bicarbonate (Dammon, 1997).

2.1.2. The preparation of the eluent solution required using an analytical balance, to weigh 29.68 g of sodium bicarbonate and 18.48 g of sodium carbonate which were transferred to a 500 mL volumetric flask.

2.1.3. Deionized water was then sparged for 15 minutes to eliminate any excess carbon dioxide in solution and was then used to fill the flask and create a 560 mM sodium bicarbonate and 440 mM sodium carbonate stock solution.

2.1.4. This stock solution was then capped and stored for later use.

2.1.5. 40 mL of the stock solution were diluted in a 2000 mL volumetric flask using sparged deionized water and mixed well to create a 11.2 mM sodium bicarbonate and 8.2 mM sodium carbonate solution.

2.1.6. This solution was then diluted with deionized water 1:9 in order to create a final eluent concentration of was 1.12 mM sodium carbonate and 0.82 mM sodium bicarbonate.

2.1.7. Regenerent concentration, also suggested by Mr. Dammon and again consistent with other ion chromatography methods, used 25 millinormal sulfuric acid. This was generated by combining 1.5 mL of sulfuric acid with 2 liters of deionized water (Dammon, 1997).

2.2. Ion Chromatograph Conditions:

2.2.1. Column: Dionex AS4A Anion Separator Column, 10 um packing material, 4 millimeter i.d. x 25 cm.

2.2.2. Guard Column: Dionex AG4A Guard Column, 3 millimeter i.d. x 6 centimeters.

2.2.3. Eluent: 1.12 mM sodium carbonate 0.82 mM sodium bicarbonate

2.2.4. Eluent Flow rate: 2 mL per minute

2.2.5. Suppressor: Dionex AMMS (Anion Micro-Membrane Suppressor)

2.2.6. Regenerent: 25 millinormal H_2SO_4

2.2.7. Regenerent Flow Rate: 3 mL per minute

2.2.8. Injection Volume: 20 uL

2.2.9. Detector: Conductivity, 20 microsiemens (uS). Background signal under specified conditions; between 10 to 20 uS.

2.2.10. Standard Analysis Time: 4 minutes

2.3. Rationale for Ion Chromatograph Conditions: The basic operating parameters for the ion chromatograph were determined from a variety of sources, but for the most part, the final parameters were determined by trial and error.

2.3.1. The column used in the experiment, the AS4A was chosen based upon guidance from Mr. Dammon and confirmed in the book titled Ion Chromatography by Weiss (Weiss, 1995). The AS4A is a multipurpose latex agglomerated anion exchanger that is inert and mechanically stable, has a fast exchange process for shorter analysis times, and high chromatographic efficiency. This allowed for a short standard analysis time of four minutes.

A guard column was used in order to protect the main column from any inadvertent damage.

2.3.2. Mr. Dammon (Southwest Research Institute) suggested flow rates were 2.0 mL per minute for the eluent and 2.0 mL per minute for the regenerant which is also standard procedure for working with this generation of columns and is consistent with other experimental procedures using ion chromatography (Dammon, 1997).

2.3.3. The type of suppressor used, the Anion Micro-Membrane Suppressor, is also common for working with bromide ions due to its ability to increase the ionic exchange capacity and its ease of use.

2.3.4. Injection volume was limited to 20 uL in order to avoid overloading the column.

2.3.5. An electrical conductivity detector was used because it is the universal method of detecting ionic species (Weiss, 1995).

2.4. Determining Operating Procedures: One of the parameters that was initially suggested by Mr. Dammon was to use an eluent concentration of 2.8 mM sodium carbonate 2.2 mM sodium bicarbonate instead of 1.12 mM sodium carbonate 0.82 mM sodium bicarbonate (Dammon, 1997).

2.4.1. Preliminary standards using the suggested eluent concentrations resulted in poor peak resolution between the large acetic acid peak and the bromide ion peak. Various concentration levels of eluent as well as various dilutions of 0.05 M solution of tetraethylammonium bromide were used to find an adequate resolution between the peaks. A dilution of 1:250 and an eluent

concentration of 1.12 mM sodium carbonate 0.82 mM sodium bicarbonate were found to produce the desired results.

2.4.2. Preliminary standards were also analyzed in order to determine if the amount of dimethylformamide used affected the outcome of the reaction. This was accomplished by analyzing six standards that were spiked with two different loading rates of DGBA

2.4.2.1. Three standards were analyzed each spiked with 20 mg of DGBA. Added to the standards were 1.0 ml, 6.0 ml, and 11.0 ml of dimethylformamide, respectively.

2.4.2.2. Three standards were analyzed each spiked with 60 mg of DGBA. Added to the standards were 1.0 ml, 6.0 ml, and 11.0 ml of dimethylformamide, respectively.

2.5. Determining Effectiveness of the Ion Chromatograph

2.5.1. In order to determine the effectiveness of the instrumentation, a series of analyses were performed to determine sensitivity, repeatability, and response. In order to determine sensitivity, additional dilutions were made of the working standards to include 0.498 mg/L, 4.98 mg/L, as well as 20 and 50 mg/L. Based upon the results, the limit of detection, calculated when the signal to noise ratio was approximately 3 to 1, was around 0.5 mg/L.

2.5.2. Repeatability was determined by analyzing multiple standards of 10 mg/L. When the coefficient of variation was less than 2 percent for the previous 4 analyses, then the instrument was considered "suitable" for analysis. This

procedure was repeated prior to each standard analysis to minimize instrument error.

2.5.3. The response of the instrument was determined by analyzing standards at 0.995 mg/L, 9.95 mg/L, 99.5 mg/L, and 199 mg/L. Then using Statistical Analysis Software (SAS) the data was analyzed to determine normality, homogeneity of variance and independence. Based on this information, the data best fit a lognormal distribution. This would require the use of three standards, as opposed to a point estimate to determine response each time.

3.0 Executing the Reaction:

3.1. Based upon information provided by Mr. Dammon the following procedures were followed (Dammon, 1997):

3.1.1. 0.5000 g of DGBA were dissolved into 25 mL of dimethylformamide. This was accomplished for both the 87 percent pure DGBA from TCI and for the precipitated 94 percent pure DGBA product.

3.1.2. Into 60 mL wide mouth amber jars with teflon cap, respectively added 0.025, 0.100, 1.00, 3.00, and 5.00 mL of dissolved DGBA into five different jars. This process was repeated four times for a total of 20 jars. Both the 87 percent pure DGBA from TCI and the precipitated DGBA product were used for a total of 40 jars. The resulting loading rates were distributed as follows:

- Four standards of 0.939 mg of DGBA (pure product)
- Four standards of 3.756 mg of DGBA (pure product)
- Four standards of 37.56 mg of DGBA (pure product)

- Four standards of 112.68 mg of DGBA (pure product)
- Four standards of 187.8 mg of DGBA (pure product)
- Four standards of 0.894 mg of DGBA (unpure product)
- Four standards of 3.576 mg of DGBA (unpure product)
- Four standards of 35.76 mg of DGBA (unpure product)
- Four standards of 107.28 mg of DGBA (unpure product)
- Four standards of 178.8 mg of DGBA (unpure product)

3.1.3. The jars were stored in their original shipping box inside a dark cabinet at room temperature.

3.1.4. Prepared 0.05 molar tetraethylammonium bromide in glacial acetic acid. This was accomplished by adding 10.50 g of tetraethylammonium bromide into 1 liter of glacial acetic acid. The solution was mixed well using a sonicator. Set aside for 30 minutes to equilibrate with room temperature. This solution was stored in a dark cabinet at room temperature.

3.1.5. To each of the 40 jars, 20 mL of 0.05 molar tetraethylammonium bromide in glacial acetic acid was added as the source of bromide for the reaction.

3.1.6. 3 mL of perchloric acid were added to each jar to create an acidic environment for the reaction.

3.1.7. Each jar was vortexed and stored in their original shipping box inside a dark cabinet at room temperature.

3.2. Standard Analysis Procedures:

3.2.1. Analysis for the standards was conducted at the following times following reaction:

- 4 hours (Day 0)
- 24 hours (Day 1)
- 7 days (Day 7)
- 14 days (Day 14)
- 28 days (Day 28)

3.2.2. The following standard preparation procedures were followed for each jar creating one standard per jar:

3.2.2.1. To a 10 mL volumetric flask, added 40 uL of the reacted solution using a Hamilton 50 uL syringe (Hamilton Co., Reno, Nevada).

3.2.2.2. Added 10 mL of deionized water for a dilution ratio of 1:250.

3.2.2.3. Filled 8 millimeter by 35 millimeter glass aliquots with diluted solution.

3.2.2.4. Placed standards into the Alcott 728 autosampler

3.2.2.5. Analyzed standards

3.3. After preliminary review of the standard results, it was thought that perhaps carryover from one standard to the next was occurring. In order to determine if this was the case, an additional analysis was conducted on the 29th day with the following changes to the analytical procedure:

3.3.1. The analysis time was increased from four minutes to eight minutes per standard

3.3.2. Eluent concentrations were increased to 2.8 mM sodium carbonate 2.2 mM sodium bicarbonate during the 5^{th} and 6^{th} minutes of the analysis in order to "flush" the system.

4.0 Obtaining and determining different purity levels of DGBA

4.1. Recommendations were provided by Dr. Gary Hagnauer, polymer chemist from the U.S. Army Research Center, Aberdeen Proving Ground, Maryland, who developed the precipitation process for obtaining pure DGBA. The initial instructions were to dissolve a large amount of unpure DGBA into methyl ethyl ketone, warm to 45-50 °C, and place in a cold environment (-20 °C) for two weeks. Spontaneously, crystals of pure DGBA would form and precipitate to the bottom of the container. This process would allow for slow crystallization resulting in a more pure product.

4.1.1. In order to successfully execute the precipitation, approximately 75 mL of methanol, 25 mL of unpure DGBA, and 15 mL of methyl ethyl ketone were placed in a 150 mL beaker and heated to 50 °C. Heating was accomplished by using a water bath placed on a combination magnetic stirrer/hot plate and stirring was accomplished by using the magnetic stirrer.

4.1.2. Heating and stirring was accomplished for 10 minutes.

4.1.3. The solution was then allowed to cool at room temperature for 10 minutes followed by cooling in -10 °C refrigerator for 10 days. Crystals

spontaneously formed in the first 30 minutes at 10 °C and within a few days complete crystallization had occurred.

4.1.4. The solution was allowed to remain in the minus 10 degree centigrade environment for 10 days.

4.1.5. The precipitated crystals were filtered using a size 11 silicone treated Whatman filter paper under vacuum. The filtered precipitate was then allowed to dry at room temperature for 3 hours and then placed into a glass container with a teflon lid and stored in a dark cabinet at room temperature.

4.1.6. Purity of the DGBA, for both the precipitated and the originally supplied product, was determined by gas chromatography following the procedures from the TCI America Company in Portland Oregon.

4.1.6.1.1. The process involved measuring out 0.0999 g of the original product and 0.1019 g of the precipitated product and dissolving each into separate 10 mL volumetric flasks of toluene.

4.1.6.1.2. 2 uL of the solutions were then injected into an HP5890 Series II Plus Gas Chromatograph (Hewlett Packard, Palo Alto, CA) containing a DB-1HT column (J&W Scientific, Folsom, CA). The column was 15 meters in length by 0.25 mm by 0.10 um i.d. The initial oven temperature was 200 °C followed by a ramp of 10 degrees per minute for six minutes with a hold at 260 degrees Centrigrade for 1 minute. This was followed by a ramp of 20 degrees per minute for four minutes with a hold at 340 °C for 20

minutes. A linear velocity of 60 cm/sec, or 1.94 mL/min, and a split ratio of 50:1 was used.

4.1.6.1.3. Detection was accomplished by flame ionization.

5.0 Data Analysis:

5.1. Before statistical analysis of the data could take place, the bromide ion peak was reviewed manually for each analysis. This was done to determine if the peak had an appropriate and consistent shape, and to make sure the integration was properly accomplished. The bromide peak, when properly formed, will look like a distended gaussian curve. The peak should not "lean" to one side or the other and it should start and finish at approximately the same baseline level. In addition, the azimuth of the peak should occur at approximately the same time for each standard, especially for standards with the same concentration. Integration of the peak is also of concern. The integration should contain all of the peak and should represent the area that is different from the baseline. A review of the integration was performed using Peaknet 5.11 provided by Dionex (Sunnyvale, CA) in order to ensure that all of the peak was properly captured as well as to make sure that additional area was not taken into account.

5.1.1. Computer integration parameters were:

5.1.1.1. Peak width of 3 seconds

5.1.1.2. Threshold of 0.1 microsiemens (uS)

5.2. The results were then used to calculate of the amount of digylcidyl ether of bisphenol A in solution using the following procedure:

5.2.1. A calibration curve was developed to explain the relationship between known concentration levels and the response of the instrument. This was accomplished using the results of the three working standard concentrations (0.995 mg/L, 9.95 mg/L and 99.5 mg/L), which were then placed into SAS which automatically determined the model which explained the logorithmic relationship between the concentration levels and the results.

5.2.2. The concentration of bromide ion initially present in the TEAB solution was calculated. This required determining the amount of bromide ion present in the 0.05 M solution of TEAB in glacial acetic acid. Since the results were assumed to be distributed lognormally, the log of the result was taken and then the result was placed into the model explained using SAS to determine the concentration of bromide ion in the 0.05 molar solution of TEAB.

5.2.3. The concentration of bromide ion consumed for each individual standard was calculated. Again, since the results were assumed to be distributed lognormally, the log of the result was taken and then the result was placed into the model explained using SAS to determine the concentration of each standard.

5.2.4. The amount of bromide consumed was determined by subtracting the amount of bromide ion remaining from the amount initially present in solution.

5.2.5. In order to stochiometrically relate the amount of bromide ion consumed with the amount of DGBA present in solution, the amount of bromide ion

consumed was divided by the molecular weight of bromide (79.9). Next, it was multiplied by the molecular weight of DGBA (340) and finally, divided by the number of equivalents per mole of DGBA (2).

5.2.6. Finally, in order to determine recovery rate, the amount of DGBA determined was divided by the amount of DGBA expected and the result was multiplied by 100 to get the results in percent.

5.3. Statistical Analysis

5.3.1. Data was placed into a Microsoft Excel® program where the means, standard deviations, and coefficients of variations were calculated. Additionally, plots were made using these values.

5.3.2. Initial evaluation of the data was performed by review of simple plots which related recovery rates and time. This was used to determine if there were any outliers and to determine if there were any trends in the data. Three standards were reviewed and determined to be "outliers" due to their significant difference from the rest of the data set, and also because the initial ion chromatograph outputs suggested that there was most likely an instrumentation error (short shot) during the analysis. These standards were P-17 on day zero, P-8 on day one, and P-6 on day 7. Next, the means and standard deviations for each of the five sets of standard types for each day were calculated and plotted to determine trends.

5.3.3. Using Minitab® 11.21 (State College, PA) a linear model was developed to explain the relationship between time and recovery rate.

5.3.3.1. This was accomplished for all of the five different concentration levels.

5.3.3.2. Residuals and fits were used from the highest two loading levels of each product to ensure that the data was normally distributed, that there was homogeneity of variance, and to assess the assumption of linearity. This function was then used to explain the amount of shelf life the standard had in order to meet the requirement of a 75 percent recovery rate 95 percent of the time.

5.3.4. Again using Minitab® 11.21, multiple pair wise comparisons were conducted to determine if there was a significant difference between the recovery rates on day zero of the experiment and all of the other days the standards were analyzed.

5.3.4.1. Again, residuals and fits were used to ensure that the data was normally distributed, that there was homogeneity of variance, and to assess the assumption of linearity. These results were then used to determine the approximate date at which the recovery rates would be less than 75 percent with 95 percent confidence.

5.3.5. An attempt was made to determine if the recovery rates could be explained by a simple function of concentration in order to determine the minimum concentration that would be needed in order to obtain a certain recovery rate.

5.3.6. Determining Carryover

5.3.6.1. Using the original data, each of the sets of data, pure and unpure, were placed into two groups. Group 1 was designated as those standards analyzed immediately after blanks and reference standards were analyzed (standards 1-5 and 11-15), and group 2 were standards that had not been analyzed immediately after blanks and standard were analyzed (standards 6-10 and 16-20).

5.3.6.2. Pair wise comparisons at each concentration were performed to see if there was any statistical difference between groups 1 and 2. The pair wise comparisons were performed for both the pure and the unpure product.

CHAPTER V

RESULTS AND DISCUSSION

The purpose of the experiments performed in this project were to determine if (1) ion chromatography is an adequate analytical tool for determining ambient concentrations of reactive epoxy resins as well as (2) to determine the stability of the DGBA standards. In order to accomplish these tasks the project was accomplished in five separate procedures:

1. Setup of the ion chromatograph to determine bromine levels.
2. Determining the reagents and quantities for reaction.
3. Executing the reaction procedures.
4. Obtaining and determining different purity levels of digylcidyl ether of bisphenol A.
5. Performing data analysis.

Essentially, three types of results were obtained from these procedures; (1) results which were used to determine reaction procedures, (2) results of the purity analysis for DGBA and (3) the results obtained from the execution of sampling protocol using the reaction procedures.

Results from Reaction Procedures:

One of the most time consuming aspects of this project was the setup of the ion chromatograph in order to analyze standards for bromide. The procedure involved determining column type, eluent concentration and flow rate, reagent concentration and

flow rate, standard size, dilution ratio, as well as integration parameters. Although most of these parameters were selected based upon literature references and personal communication, other parameters were determined through trial and error. Some of these parameters, the eluent concentration and the dilution ratio, were based upon a preliminary study using three different loading rates. Dependent variables included the eluent concentration and the dilution ratio while all other variables were held constant. Figure 4 is a typical chromatograph obtained when analyzing a mixture of

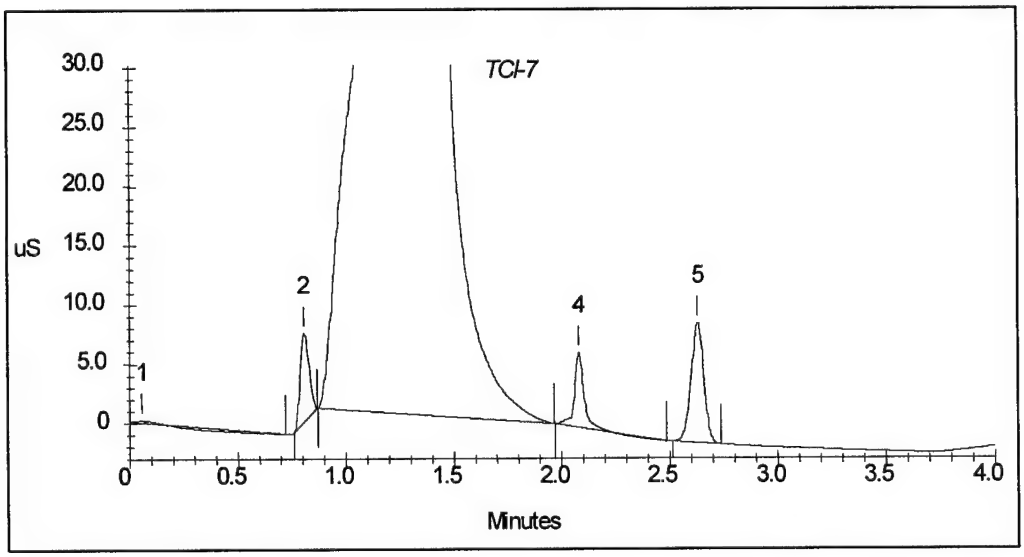

Figure 4 -- Elution profile for the IonPac AS4A separator column for bromide, peak 5, and glacial acetic acid, peak 3 (not completely shown). Eluent 0.82mM Na2CO3 + 1.12 mM NaHCO3; flow rate 2 mL/min; detection: suppressed conductivity; injection volume 20 uL

acetic acid and TEAB. Notice the large acetic acid peak (peak 3, off the scale) followed by the bromide peak, peak 5, which has a retention time of around 2.6 minutes. The purpose of the preliminary study was to determine the maximum eluent concentration and the minimum dilution ratio that would result in separation between the bromide peak and the large acetic acid peak. Improved resolution between the peaks will eliminate interference and improve analytical performance (Weiss, 1995).

The choice of eluent concentrations and dilution ratios were based upon recommendations from Mr. Mike Dammon, Southwest Research Laboratory in San Antonio Texas. Variations of each of these variables were explored in order to determine the effect upon peak resolution.

Peak resolution, as defined by Weiss' book Ion Chromatography, can be determined using the following equation:

$$R = \frac{t_{ms1} - t_{ms2}}{\frac{w_1 + w_2}{2}}$$

Equation 2 -- Determining resolution between two neighboring peaks

where:

R	Resolution
w_1, w_2	Peak widths for peak 1 and 2 respectively
t_{ms1}, t_{ms2}	Gross retention times for signal 1 and 2 respectively

Using equation 2 while varying the dilution ratio as well as the eluate strength, 10 standards were analyzed to determine the optimum resolution while obtaining an adequate peak size. The resulting peak resolutions were determined:

Table 1 -- Standards of 0.05 M tetraethylammonium bromide (TEAB) in glacial acetic acid analyzed by ion chromatography using various eluent strengths of $NaHCO_3/Na_2CO_3$ and various dilution ratios with deionized water. Flow rate for each standard was 2.0 mL/min; detection was suppressed conductivity; injection volume 20 uL.

Dilution	Eluate (%)	t_{ms1}	t_{ms2}	w_1	w_2	R	Peak Area
1000	25	0.97	1.67	0.75	0.2	1.474	8679368
500	25	1.01	1.75	1	0.2	1.233	19847300
250	25	1.09	1.85	1	0.2	1.267	40101145
1000	20	1	1.79	0.7	0.2	1.756	8800002
500	20	1.06	1.88	0.8	0.2	1.640	19790890
250	20	1.16	2.01	0.8	0.2	1.700	40493590
1000	15	1.05	1.97	0.7	0.2	2.044	9676260
500	15	1.14	2.1	0.8	0.2	1.920	20739330
250	15	1.27	2.27	1	0.2	1.667	43644670
250	10	0.82	2.74	1.2	0.2	2.743	41793210

Table 1 shows that as the dilution rate decreases from 1000 to 250, the peak area and the peak size increase. Therefore, as the dilution ratio goes down, the sensitivity of the procedure, or the ability to detect smaller amounts of bromide, increases. Also, as the elution strength decreases, the resolution between the glacial acetic acid peak and the bromide peak increases. According to Weiss, a resolution of 2 is considered adequate for chromatographic procedures (Weiss, 1995). Therefore, based upon the results from the preliminary experiment, a dilution ratio of 1:250 was chosen as well as an eluent strength of 10 percent, or a concentration of 1.12 mM sodium carbonate and 0.82 mM sodium bicarbonate.

A second preliminary study was performed using the information obtained through the first preliminary study in order to determine if the amount of dimethylformamide (DMF) used affected the recovery rates of the standards.

Table 2 – Recovery rates for standards reacted with variable amounts of dimethylformamide. Dilution ratio 1:250; eluent 0.82mM Na2CO3 + 1.12 mM NaHCO3; flow rate 2 mL/min; detection: suppressed conductivity; injection volume 20 uL

Sample #	DMF	Epoxy (mg)	Expected (mg)	Recovery (%)
1	1	18.932	20	94.66
2	6	20.023	20	100.12
3	11	21.060	20	105.30
4	1	68.887	60	114.81
5	6	62.170	60	103.62
6	11	61.887	60	103.14

Based upon the results shown in Table 2, neither the amounts of DMF used, the elution strength or the dilution ratio affected the recovery rates of the standards. In this case, all of the recovery rates, with the exception of standard 4, are very close to 100 percent with

some variability as a result of either instrumentation, or perhaps a minor effect as a result of the difference in concentration of DMF.

Determining the purity of Diglycidyl Ether of Bisphenol A

One of the most important parts of this experiment was to look at the differences between the 87 percent pure liquid product and the more pure precipitated product. Based upon the results using gas chromatography, the precipitation process resulted in a more pure product than the purchased DGBA product. Determined purity for the purchased product was 89.4 percent and for the precipitated product was 93.9 percent. Gas chromatograph results from TCI America showed 88.4 percent purity when the standard was analyzed, using the same procedures, with a similar column, but a 5 percent dilution of the product in toluene. The same loading of product was tried, but resulted in too high a loading rate on the column, as seen by tailing, and consequently poor results. Overnight "baking" of the gas chromatograph was performed in order to mobilize the DGBA off of the column. Using a 1 percent dilution of DGBA in toluene, a result of 89.4 percent purity was obtained, which was similar to those obtained by TCI America for the unpure product. Additionally, the peaks obtained were not tailing, demonstrating that the column was no longer overloaded (Rubinson & Rubinson, 2000). See appendix II for the 1 percent dilution chromatographs.

Results from Experimental Protocol

The first goal of this project was to determine if ion chromatography, used as an analytical tool, is adequate for determining ambient concentrations of reactive epoxy resins. Recovery rates were calculated in order to measure the effectiveness of this method. Recovery rates are calculated by dividing the amount of epoxy resin calculated

using experimental methods by the amount of epoxy resin measured and placed into the standard. Four standards at each loading rate for both the "pure" precipitated product and the "unpure" original product were prepared for a total of 40 standards. Standards were identified as P-# for "pure" product and T-# for the "unpure" product. Standards were analyzed in order from P-1 to P-20 followed by T-1 to T-20 for each day the standards were analyzed.

The second goal of this project was to determine standard stability to determine how long the standards were viable according to standards set by NIOSH. Standards were analyzed on the day of reaction (day 0), 1 day after reaction, 7 days after reaction, 14 days after reaction, 28 days after reaction, and 29 days after reaction. Standard results were then compared from day 0 to the day of interest to determine the amount bromide ion consumed during the reaction. With the exception of the analysis of standards for day 29, laboratory conditions and analysis procedures were the same. On day 29, increased analysis times and an increase in eluent strength after the bromide peak came off the column was performed. See page 31 in the methods section of this paper for the change in procedures for day 29.

Using Ion Chromatography as an Analytical Tool

In order to analyze the data using statistical methods, "outliers" were first determined and removed from the data set. These outliers were selected upon review of the chromatograph outputs and by comparison with standard analyzed on different days, as well as through comparison of standards with the same loading rates. For example, the results from analysis of P-17 on day 0 was identified as a potential outlier because of the poor quality of the chromatograph output. When P-17 was analyzed on day 1, the

amount of bromide found was significantly larger than on day 0 and it was consistent with the other standards at the same loading level on both day 0 and day 1. Therefore, P-17 on day 0 was selected as an outlier. Two other standards were selected using the same criteria of (1) unusual or poor chromatograph output, and (2) comparison with other standard analyses for that standard. The three standards that were chosen to be removed as outliers were P-17 on day zero, P-8 on day one, and P-6 on day 7.

Once the outliers were removed, examination of the efficacy of the method was examined by reviewing recovery rates for each of the loading levels. According to the

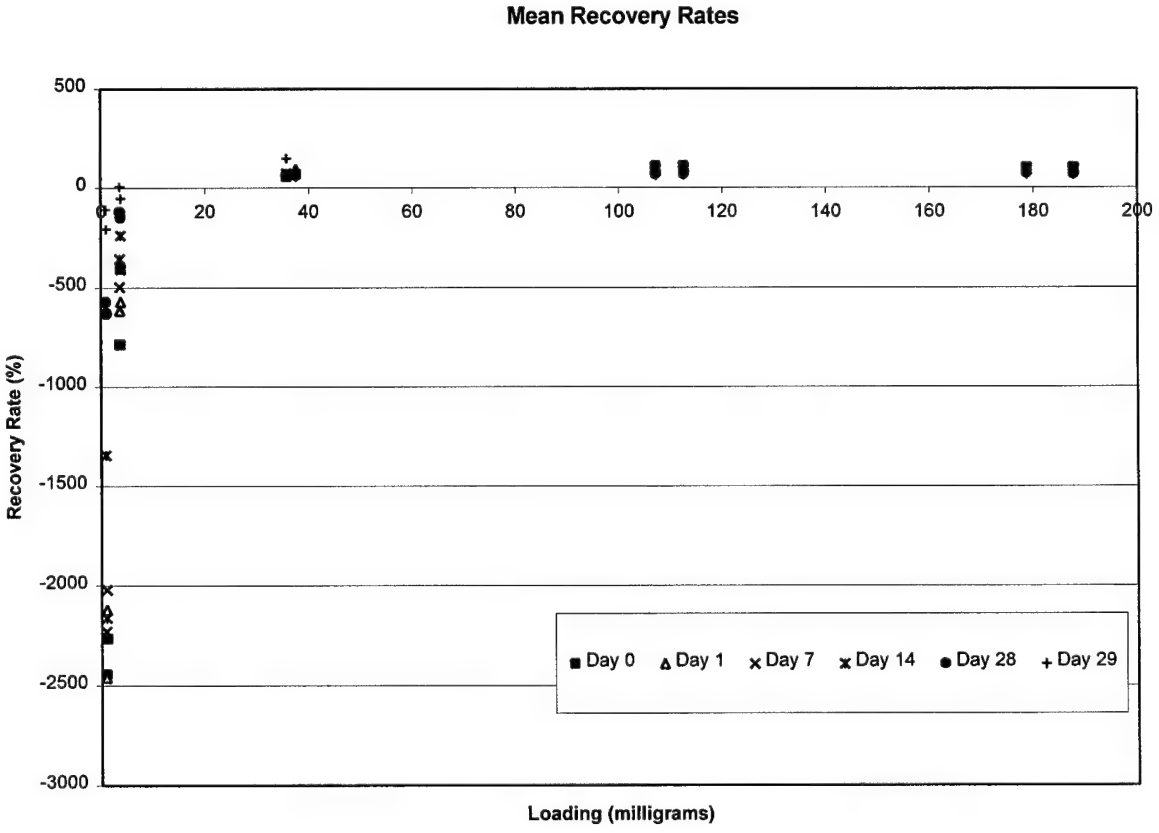

Figure 5 -- Mean Recovery Rates, in percent, as a function of loading of DGBA standards, in milligrams. Rates are plotted for time periods after the initial reaction procedure (day 0). All loading rates included.

NIOSH Method of Analytical Methods, a method must have a recovery rate of at least 75 percent 95 percent of the time (National Institute for Occupational Safety and Health, 1994). Therefore, an initial review of the data involved visual inspection of the plots included relating mean recovery rates to standard loading for each of the standard analyses. The most striking finding is the "negative" recovery rates for the very low loading of DGBA with improved performance at the middle loading, and very good results for the highest two loading levels. For example, in Figure 5, at a loading rate of approximately 4 milligrams of DGBA, mean recovery rates ranged from almost "negative" 800 percent to 0 percent.

The "negative" recovery rates are only possible because bromide is used as a surrogate for DGBA and as a result, if more bromide is measured than expected, then a "negative" recovery rate results. Figure 5 also shows that there is a large amount of variation in recovery rates at the lower loading rates of DGBA and not as much variability of recovery at the higher loading rates.

In order to more completely visualize what is happening at the higher loading rates, Figure 6 was prepared. Figure 6 shows that above 100 milligrams of DGBA, recovery rates were both consistent, with little variation, and approximately 100 percent for the standard analyzed on day 0. However, there is a U-shaped pattern to the results. At the lower loading rate in Figure 6, the recovery rates are well below 100 percent for all days analyzed. The middle loading rate has recovery rates over 100 percent for the first few days and then less than 100 percent for the rest of the days. The highest loading rate

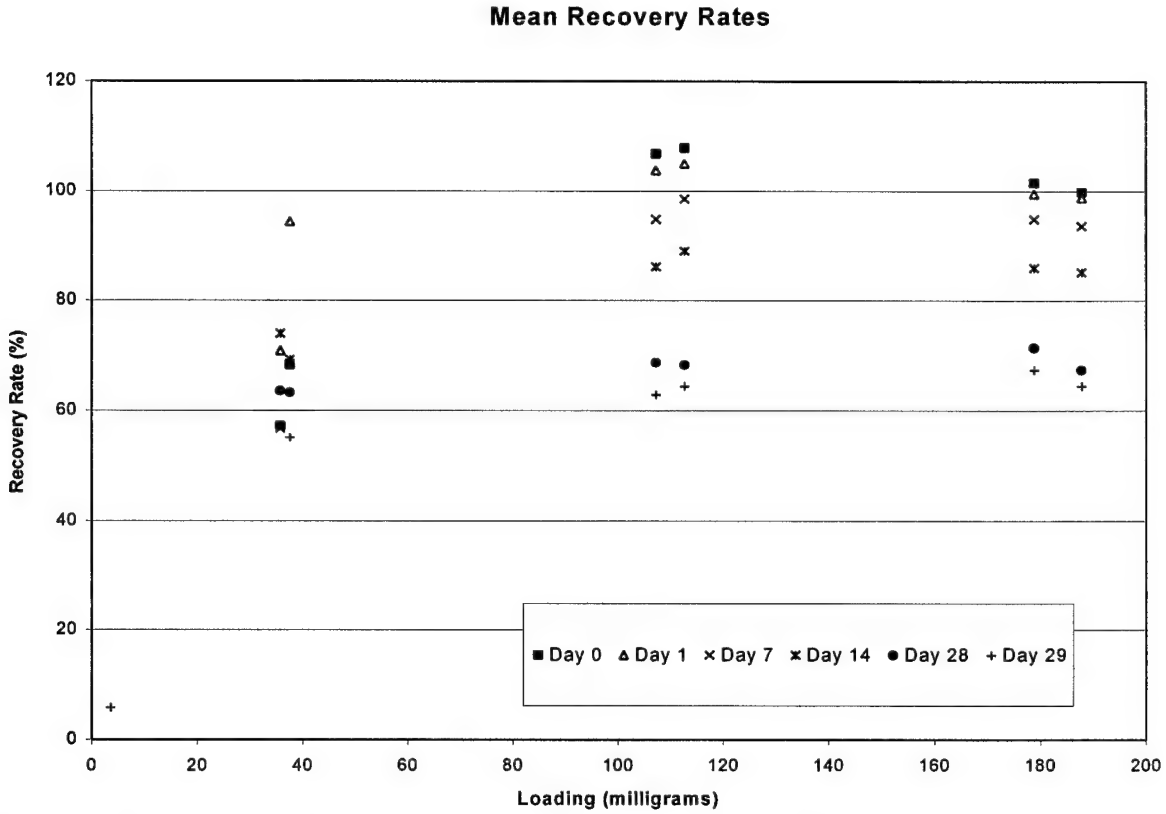

Figure 6 -- Mean Recovery Rates, in percent, as a function of loading of DGBA, in milligrams. Rates are plotted for time periods after the initial reaction procedure (day 0). Only highest three loading rates

has recovery rates of around 100 percent for the first few days and then less than 100 percent for the remainder of the days analyzed. This shows that as time progresses, the recovery rates decrease.

Table 3 -- Mean recovery rates in percent of DGBA analytically determined compared to the amount of DGBA spiked into the standard

Loading Level	Day 0	Day 1	Day 7	Day 14	Day 28	Day 29
0.89	-2444.06	-2459.83	-2228.63	-1344.81	-573.12	-110.08
0.94	-2265.14	-2118.80	-2020.03	-2159.40	-629.15	-207.73
3.60	-787.35	-615.69	-497.77	-358.40	-121.55	5.82
3.80	-405.46	-572.01	-409.83	-239.47	-146.87	-53.00
36.00	57.23	70.87	56.79	73.97	63.61	148.52
38.00	68.30	94.38	68.58	69.25	63.29	55.11
107.00	106.81	103.76	94.82	86.12	68.74	62.92
113.00	107.85	104.97	98.53	89.02	68.29	64.47
179.00	101.54	99.50	94.84	85.98	71.43	67.42
188.00	99.81	98.73	93.59	85.16	67.44	64.52

Table 4 – Standard deviations for the mean recovery rates obtained

Loading Level	Day 0	Day 1	Day 7	Day 14	Day 28	Day 29
0.89	855.28	1313.41	2013.44	1810.84	1934.53	2219.31
0.94	983.88	951.59	1105.90	1128.11	1214.76	1352.41
3.60	399.02	360.96	345.70	282.45	305.43	261.58
3.80	203.56	343.71	347.81	197.51	209.79	239.67
36.00	29.52	23.55	25.40	35.32	35.36	191.17
38.00	34.69	6.64	18.15	22.23	11.93	13.60
107.00	3.04	5.24	3.61	5.53	2.89	2.02
113.00	4.69	5.91	4.97	10.18	6.91	2.25
179.00	1.82	2.23	1.62	1.54	1.49	2.68
188.00	2.15	1.62	1.22	2.86	2.30	5.77

Table 5 -- Coefficients of variation for the mean recovery rates obtained

Loading Rate		Day 1	Day 7	Day 14	Day 28	Day 29
0.93	-0.35	-0.53	-0.90	-1.35	-3.38	-20.16
0.96	-0.43	-0.45	-0.55	-0.52	-1.93	-6.51
3.72	-0.51	-0.59	-0.69	-0.79	-2.51	44.94
3.84	-0.50	-0.60	-0.85	-0.82	-1.43	-4.52
37.2	0.52	0.33	0.45	0.48	0.56	1.29
38.4	0.51	0.07	0.26	0.32	0.19	0.25
111.6	0.03	0.05	0.04	0.06	0.04	0.03
115.2	0.04	0.06	0.05	0.11	0.10	0.03
186	0.02	0.02	0.02	0.02	0.02	0.04
192	0.02	0.02	0.01	0.03	0.03	0.09

In addition to the mean recovery rates, as shown in Table 2, the standard deviation, shown in table 3, and coefficients of variation, shown in Table 4, for each of the five sets of standard types were calculated. The results concur with the visual assessment of the data. High variability exists at the lower loading levels, and low variability at the higher loading levels. However, the variability is fairly consistent between each of the different standard analyses suggesting repeatability for the data. Since mathematically the standard deviation and coefficients of variation are related, they were also high for the

lower loadings and low for the higher loadings. Compared to the results obtained during the experiments conducted by Dr. Herrick, these results are unfavorable. Dr. Herrick was able to confidently attain recovery rates around 100 percent for standards containing as little as 0.5 milligrams of DGBA. Obviously, the standards spiked with 1 milligram of DGBA did not have recovery rates which were even close to those obtained by Dr. Herrick. However, the reported coefficients of variation for Dr. Herrick's experiment was 0.06 (Herrick & Smith, 1987). This experiment found similar results at the higher loading levels with more variation at the lower loading levels. See appendix III for individual standard results.

Since the main reason for this experiment was to show that ion chromatography was at least as sensitive as normal pulse polarography, rationale for the differences in results requires explanation. Two possibilities include poor mixing and acetic acid peak interference.

The first possibility, poor mixing, would result in poor recovery rates because the bromide would not interact with the DGBA and therefore, would not get consumed. However, this does not explain why there is an increase in bromide levels, and it also doesn't explain why there are consistently negative recovery rates for all of the low loading standards. If poor mixing did occur, large variations in the results would be likely to occur since some standards statistically would be mixed better than others. Since there are some high "positive" results, poor mixing cannot be ruled out entirely, but it is more likely that the few "positive" results are a consequence of either instrument error (short shots) or a result of poor analytical technique. Therefore, poor mixing should be ruled out as a reason for the large difference in results. Instead the most likely reason

for the poor recovery rates is carryover, or column retention of the bromide from one standard to the next.

Carryover is the most likely scenario and there are multiple levels of evidence that support this idea. According to Rubinson & Rubinson, the stationary phase of a column can be overloaded when "so much material is bound on the stationary phase in the region of high concentration that no more can do so" (Rubinson & Rubinson, 2000). The strong interaction leads to tailing and can cause standard contamination in subsequent analyses, referred to in this paper as carryover. A review of the results suggests that one needs to explain where the additional bromide is coming from. Simple mass balance operations make it difficult to see more bromide after the reaction than was totally added. Secondly,

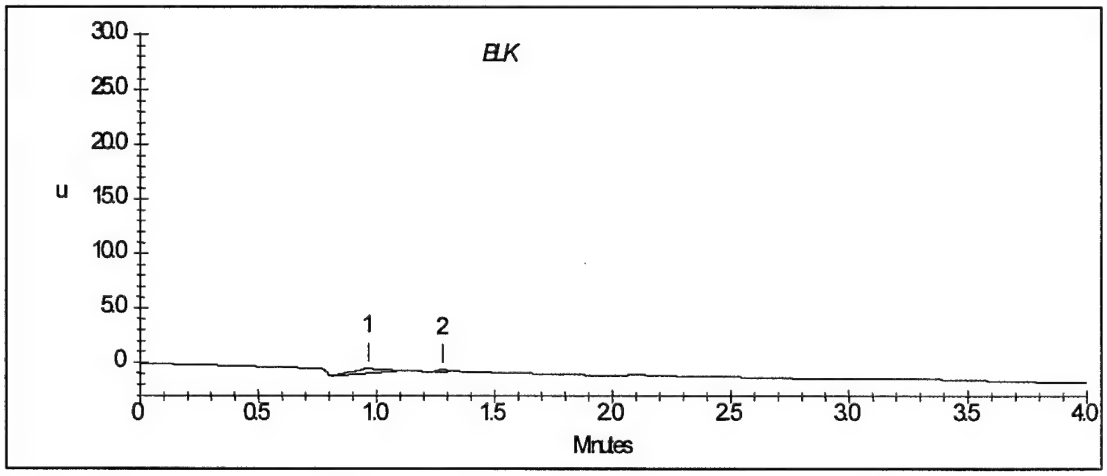

Figure 7 --Full scale elution profile for the IonPac AS4A separator column for a blank standard. Eluent 0.82mM Na2CO3 + 1.12 mM NaHCO3; flow rate 2 mL/min; detection: suppressed conductivity; injection volume 20 uL

although the use of blanks were employed in order to visually catch "carryover", initial review of the blank standards did not show evidence of carryover. This can be seen in Figure 7 showing that at approximately 2-2.5 minutes, where bromide typically comes off of the column, no peak can be seen. However, when the plot is magnified, there was evidence of bromide carryover, as shown in Figure 8. Third, the sequencing of the

standards indicate there is more effect, or more carryover, the farther away a standard is from a blank standard. In this experiment, the sequence of standard analysis was to

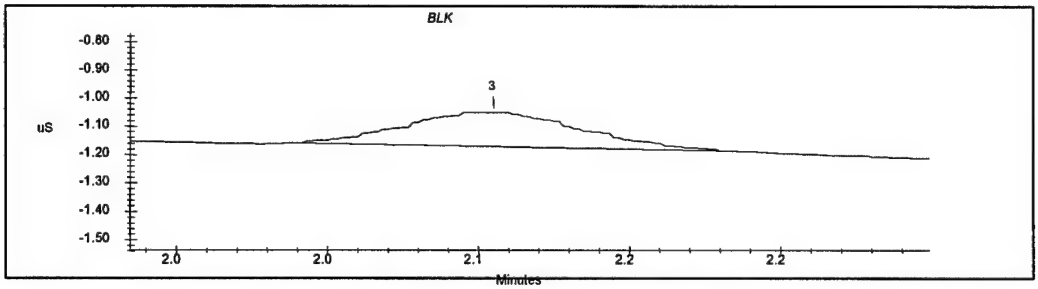

Figure 8 -- Magnified elution profile for the IonPac AS4A separator column for a blank standard. Eluent 0.82mM Na2CO3 + 1.12 mM NaHCO3; flow rate 2 mL/min; detection: suppressed conductivity; injection volume 20 uL

analyze 10 standards, or two sets of loading levels, followed by a set of reference standards and a blank. Simple observation of the data showed that standards analyzed immediately after a blank had less of a carryover effect than standards analyzed without a blank. Additionally, it showed that the carryover effect is reduced at the higher loading levels, although there still is some effect on the data. Also, the higher recovery rates at around the 120 milligram level as compared to the 200 milligram level are a result of carryover.

In order to test whether there was statistically significant differences in results between standards analyzed near blank standards and those analyzed farther away from blank standards, analysis of variance was used. Using Minitab® 11, analysis of variance was used to determine if there was statistically significant difference between the analyses that occurred immediately after standards and blanks were analyzed (group 1), and those that were not analyzed after standards and blanks were analyzed (group 2).

Table 6 – Analysis of Variance for groups 1 and 2 performed at the 95 percent confidence level

Loading (mg)	Difference in Means	P-value (Parametric)	P-value (Non-Parametric)
0.89	2609	<0.000	<0.000
0.94	1730	<0.000	0.003
3.6	547.9	<0.000	0.001
3.8	412.6	<0.000	0.001
36	12.3	0.716	0.001
38	24.63	0.003	0.003
107	4.45	0.539	0.525
113	7.39	0.331	0.204
179	0.44	0.939	0.939
188	2.68	0.667	0.525

The results, as shown in Table 6, show significant differences between the two groups at the lower loading levels. This is because the effect of carryover is greatest at the lower loading levels in group 1 which were analyzed soon after blanks and reference standards were analyzed. At the higher loadings, the groups were not significantly different from one another because they were analyzed under almost the same set of conditions. They were analyzed near other standards, as opposed to references or blanks, and the results were not affected by the use of blanks and reference standards. In fact, at the higher levels, especially at the 179 mg loading level, there were no significant differences between the two groups. This supports the idea that carryover not only had a direct effect upon the results, but it also influenced the variation in the data. The effect that carryover had on the means is straightforward. Especially at the lower concentrations, the results were negatively biased due to additional bromide from previous analyses. This effect was also seen at the higher concentrations, but to a lesser degree. Additionally, carryover had an effect upon the variation in the results. Since all of the standards were not analyzed under the exact same conditions (i.e. some were analyzed closer to blanks than

others, some were analyzed earlier in the entire standard analysis), then the change in the amount of carryover, as evidenced by the differences between groups 1 and 2, caused variation in the results.

The comparisons were also performed non-parametrically, using the Kruskal-Wallis test, as well as parametrically, using analysis of variance. With the exception of the 36 mg loading level, all of the results are very similar. The difference in this particular loading level is a result of a very high result on day 29 of analysis. Complete analysis of variance results can be found in appendix IV.

Unfortunately, the preliminary studies did not show this carryover effect to this magnitude. This may be due to a couple of reasons. One, the amount of standards analyzed during the preliminary studies were not as large as the standards analyzed during the experiment. The second reason that carryover was not seen, was that most of the standards analyzed previous to the experiment were analyzed at higher eluent loading levels as recommended by Mr. Mike Dammon of the Southwest Research Institute. The third reason may be due to the fact that magnification of the data from blank standard analyses were not conducted. Since there was not any evidence of carryover at the time, the need for performing this procedure seemed unnecessary.

Standard Stability

The second goal of the project was to determine the stability of the standards over time. In order to determine if there were differences in recovery rates between the sampling analyses, and thus determine the long term stability of the standards, one way analysis of variance (ANOVA) was performed. Using Minitab® 11, Tukey's pair wise comparisons, using day as the dependent variable, between each of the standard analyses

were accomplished for each of the five different loading levels. Statistically significant differences, at the 95 percent confidence level, were seen only at the 120 and 200 milligram loading levels for the precipitated product and at the 40, 120 and 200 milligram levels for the unpure product. This is consistent with the initial visual inspection of the data as well as the low standard deviations at the higher loadings and the wider variation at the lower loadings. See appendix V for the complete listing of pair wise comparisons and their statistical significance.

Models were then generated using linear regression to explain standard stability using day as the 'X' or dependent variable, and loading rate as the 'Y' or independent variable. Loading levels are defined as "RecX" where X is the loading level used. The resulting models and their R-squared values are listed in table 7.

Table 7 -- Regression models for determining standard stability of DGBA

Loading	Model	R-Squared
Precipitate, 188 mg	Rec188 = 101 - 1.20 Day	96.1%
Precipitate, 113 mg	Rec113 = 108 - 1.44 Day	90.3%
Precipitate, 38 mg	Rec38 = 77.8 - 0.661 Day	13.8%
Precipitate, 3.8 mg	Rec3.8 = - 497 + 14.3 Day	33.1%
Precipitate, 0.94 mg	Rec0.94 = - 2413 + 64.5 Day	36.0%
Unpure, 179 mg	Rec179 = 102 - 1.12 Day	97.8%
Unpure, 107 mg	Rec107 = 106 - 1.40 Day	95.2%
Unpure, 36 mg	Rec36 = 56.5 + 1.67 Day	6.3%
Unpure, 3.6 mg	Rec3.6 = - 696 + 22.8 Day	45.9%
Unpure, 0.89 mg	Rec0.89 = - 2549 + 77.6 Day	26.3%

This table shows the similarities between the results of the pure product and the unpure product. The higher the loading, in this case, the higher the R-squared value, demonstrating that it is a better linear model. In fact, good linear models were developed at the two highest loading levels with R-squared values over 90 percent. Poor linear models were developed at the lower three loading levels. As a result, R-squared values

did not exceed 50 percent for any of the lower loading levels, and as a consequence of the poor results, they did not explain how long the standards remained viable. This is consistent with the recovery rates obtained, especially considering the large variability that needed explanation at the lower loading levels.

Using the residuals from the linear models at the two highest loading levels, standardized residuals, and the fitted values from the regression model previously described, the assumptions of normality, linearity, and homogeneity of variance were tested. For the precipitated product, once the outliers were removed, all of the assumptions were met (see appendix VI for results). The Ryan-Joiner normality test showed linear response ($P > 0.10$), the test for homogeneity of variance did not show any trends, and the test for linearity also did not demonstrate any trends. For the unpure product, the removal of any outliers was not necessary in order to meet all of the assumptions (see appendix VII for results). Like the pure product, the Ryan-Joiner normality test showed linear response ($P > 0.10$), the test for homogeneity of variance did not show any trends, and the test for linearity also did not demonstrate any trends. Therefore, transformation of any portion of the data was not necessary.

Using the linear regression models from Table 7, the amount of time that standards were considered to be stable, based upon the NIOSH requirement of a 75 percent recovery rate 95 percent of the time, were determined (National Institute for Occupational Safety and Health, 1994). The last day at which recovery was still expected to exceed 75 percent was determined to be:

Table 8 – Calculated maximum time DGBA standards remained stable using guidelines from the NIOSH Manual of Analytical Methods

Loading	Day
Precipitate, 188 mg	20
Precipitate, 113 mg	20
Unpure, 179 mg	22
Unpure, 107 mg	20

More complete results can be found in appendix VI and VII.

Since it is obvious that reduction in standard stability is occurring over time for each of the loading levels, one of the important questions that must be answered is "Where does the bromide go?" It is well recognized that bromide is unstable, especially in the presence of light. In this experiment, 60 milliliter amber jars were used in order to reduce the effect of light on bromide stability. Additionally, the standards were maintained in a dark location to prevent light exposure to the bromide ions in solution. Despite these efforts, bromide decay still occurred, although without analysis of control standards, the actual effect of using amber jars and storage away from the light cannot be determined.

Limitations of the Experiment

One of the limitations of this experiment was the inability to obtain pure diglycidyl ether of bisphenol A. Since no literature could be found describing the procedure for purifying DGBA, Dr. Herrick was contacted to determine where pure DGBA could be found. Dr. Herrick recommended contacting any of the chemical manufacturers of epoxy resins. Shell and Cieba-Giegy did not sell or use pure DGBA and therefore were unable to sell any pure product. Dow Chemical, on the other hand, used pure DGBA, but was unwilling to sell any of the pure chemical for undisclosed

reasons. A polymer chemist at one of Dow Chemical's suppliers, DAJAC Polymer/Monomer, was unsure of even how to proceed in purifying DGBA. Dr. Herrick also recommended contacting Dr. Gary Hagnauer, a polymer chemist at the Army Research Laboratory, who provided him with the pure chemical when he conducted his work in 1987. Dr. Hagnauer had not performed this process in over 15 years, however, he provided the basic instructions for precipitation of the DGBA for this experiment. Because it had been so long since he had performed the process himself, he was unsure of the specifics in order to ensure purity. He did say that the most important part of the precipitation process was to have a slow crystallization procedure to ensure the highest purity possible. Initially the process only involved the use of methyl ethyl ketone to form the precipitate. Without success, Dr Hagnauer suggested adding a small percentage of methanol in order to help the process along. Ultimately, a significant portion of the mixture included methanol in order to have precipitation occur. Although crystallization for the successful experiment was slow, taking a few days, Dr. Hagnauer thought that the procedure might take a few weeks. Therefore, the effectiveness of purification is perhaps questionable. Although there was a change in purity of DGBA, 89.4 percent to 93.9 percent, a greater difference in purity would have been preferred in order to effectively determine the differences, if any, in the efficiency of reaction with the "pure" and "unpure" products.

Despite the fact that there were still 6 percent impurities in the precipitated product which is different from the experimental conditions used by Dr. Herrick, it is very unlikely that the differences in the data from this experiment and from Dr. Herrick's work can be attributed to the difference in purity of product. Since other studies have

shown the effectiveness of the stochiometric reaction between bromine and DGBA to determine epoxy resin content, it is more likely that there is another reason for the discrepancy.

Another limitation, and a source of error in this experiment were the large number of transfers of chemicals that took place. Unlike some industrial hygiene methods where standard preparation is one or two steps, the addition of dimethylformamide, perchloric acid, acetic acid and DGBA must be accomplished prior to the reaction with a dilution of the chemical after the reaction. Since the total error of the experiment is a result of the sum of the root mean square of the individual errors, then each step adds a little more human and mechanical error to the experiment. Since the data were consistent from one loading to the next, it is unlikely that these errors played a significant part in the results of the experiment.

Another limitation of the experiment was the number of standards taken at each loading level. Although effort was taken to ensure enough standards were taken to observe significant differences between loading levels, almost every study has this limitation. Specifically, in this case, not enough standards were taken at lower loading levels in order to determine if there was a significant difference between the different time periods examined. Furthermore, an increase in standard size would have decreased the amount of variability seen in the results.

One of the points that should be taken from this study is that preliminary work is very important in any research project and should not be overestimated. Initially, this method seemed to be very straightforward with little risk of failure. Attempts were made to ensure successful implementation of the project. These included determining the

optimum eluent loading through trial and error. Different dilutions were examined in order to ensure a significant amount of bromide could be detected by the ion chromatograph. Finally, the standards were not analyzed until there were assurances that there was a difference between the original DGBA product and the purer precipitated DGBA product. However, a trial analysis using all 40 chemicals was not accomplished because of the amount of preparation time for each standard. Consequently, the obvious effect of carryover was not investigated completely. As a result, poor recovery rates at the lower loading levels occurred. What has been learned is that research is not just science, it involves the application of science which in some ways is an art. If this experiment were to be repeated, more preliminary work should be accomplished prior to beginning.

Future Work

As in most research, in an attempt to answer one question, usually many other unanswered questions become apparent. This is certainly the case of this research project. In fact, there are many possible avenues that should be explored in order to perfect this method as an analytical tool.

One avenue of future study would be to determine the effect of varying the loading of tetraethylammonium bromide (TEAB). Per Mr. Mike Dammon, a loading of 0.05 molar TEAB in glacial acetic acid was used. Because the acetic acid peak was very large in comparison to the bromide peak, extra effort was made to ensure an eluent loading that separated the acetic acid peak from the bromide peak. As a result of the low eluent loading, some chemicals, to include bromide, might have stayed on the column

longer than necessary resulting in the "carryover" effect. One way to mitigate the effects of carryover is to alter the concentration of TEAB and to observe the effects.

In the same regards, varying the molar ratio of TEAB to DGBA could be explored. The suggested molar ratio by Selig and Crossman and confirmed by Dr. Herrick is a 3:1 molar ratio of TEAB to DGBA (Selig & Crossman, 1971). Unfortunately, in the field such neat molar ratios are not always possible. In this experiment, various molar ratios, were used in order to explore the effect of varying molar ratios. However, because it was not the only independent variable used in the experiment, and due to the poor recovery rates at lower loadings, explanation of its effect upon the results is not possible.

Table 9 -- Molar ratio of bromide to DGBA at each of the loading levels

Loading (mg)	Millimoles of Bromide Used	Molar Ratio
0.9	1	366
3.8	1	87
38	1	8.7
113	1	3.0
188	1	1.7

Another means of determining the cause of poor recovery rates would be to use varying concentrations of dimethyformamide. Dimethylformamide is used as the solvent that stops DGBA from reacting with itself via a curing agent. Although it is unlikely that DGBA reacted with the container or other chemicals when low amounts of DMF were used, it should be examined. This is definitely a concern when applying this method to field work where varying amounts of DMF will occur as a result of the vaporization of DMF while using an impinger. Although preliminary work was performed during this

experiment, a more detailed review should be performed in order to rule out any uncertainties about this process.

One experimental method that could be explored is the extension of standard analysis time to ensure the entire amount of chemical is "flushed" from the column prior to analyzing another standard. Although this was explored in standard analysis day 29 with the standard analysis time doubled from 4 minutes to 8 minutes, additional work could be done in combination with modification of other variables to ultimately ensure "clean" analysis each time.

One of these "variables" is the choice of eluent concentration. Based upon the guidance from Mr. Mike Dammon, a stronger concentration of eluent was recommended. Preliminary work showed poor resolution between the acetic acid peak and the peak of interest, bromide, when his recommendations were followed. Instead, a weaker concentration of eluent was used, and perhaps, the weaker concentration is responsible for the poor results at low concentrations.

Mr. Dammon also suggested using a dilution ratio of 1:500 (standard:water) for standard preparation as opposed to the 1:250 employed in this experiment. Again, preliminary work was accomplished and a ratio of 1:250 was determined as the best ratio in order to have a wider range of detection during the experiment while ensuring resolution between the acetic acid peak and the bromide peak. Future studies could investigate different dilution ratios in order to determine the optimum dilution ratio; one that would eliminate carryover while providing the greatest amount of sensitivity.

Future work should also entail an attempt to acquire as pure as possible DGBA. Since the purity of the DGBA that Dr. Herrick worked with in his experiment was not

reported, the precipitated chemical used in this experiment cannot be compared directly. Instead, additional work could be accomplished to "perfect" the precipitate process, or more research could be conducted to find a seller of the more "pure" chemical product. Also, lobbying of Dow Chemical could be accomplished in order to obtain their more pure form of DGBA.

Whether or not more "pure" product is obtained, there is additional work that can be accomplished with mixtures of DGBA. Simple studies could include looking at the differences in results when using different percentages of DGBA. For example, this could include looking at the differences between percentages such as 15-25 percent DGBA and pure product since these lower percentages are more representative of industrial use chemicals. Additionally, different mixtures of DGBA and typical epoxy resin constituents such as diluents and curing agents could be explored to see their effect on recovery rates.

An area of the experiment that could be compared is the type of container for maintaining the standards as well as the type of environment in which they are stored. Some standards could be stored in a normal laboratory setting at room temperature, others in the refrigerator or freezer, and each location could have sets of standards stored in amber jars as well as standards stored in clear glass containers.

Other suggested studies include the applicability of this method to field work. Aerosol samples could be collected either directly in the industrial setting and analyzed using this method, or samples could be generated in a chamber, collected and analyzed using this method. However, improvements in the details of the method are necessary

before taking the next step in the process of developing this method for industrial application.

Although most of the possibilities for future work regarding epoxy resins discussed so far have centered around the analytical method, there are still many gaps in toxicology and epidemiologic assessment information that should be scientifically determined in order to support, or perhaps even refute, the health reasons for this project.

One of the areas that should be explored is the effect of inhalation exposure to aerosols of epoxy resin. To date, there have not been any toxicological studies using animals or humans to assess the health effects of inhalation exposure to DGBA or epoxy resins. The reason for the lack of information is due to the fact that DGBA does not have a measurable vapor pressure and consequently, most researchers assume that inhalation exposure is negligible. Since most workers that use paints apply the paint by aerosolizing the mixture, inhalation exposures are not only possible, but without respiratory protection, they would be typical. Therefore, toxicological studies should be performed which measure the effect of inhalation exposure to aerosol DGBA.

Another avenue that can be explored more fully is the epidemiology of DGBA exposure and workers. In 1990, Jolanki put together a complete epidemiologic review of the effects of epoxy resin exposure (Jolanki et al., 1990). She did an excellent job of looking completely at all components of the epoxy resin mixture in order to understand which component was most likely to be the cause of adverse health effects. Since that time, many more papers have been published relating the adverse health effects to exposure, but since a sampling method does not exist for determining exposure levels to DGBA, the relationship between amount of exposure and the health effect has not been

fully developed (Angelini et al., 1996; Conde-Salazar, Gonzalez de Domingo, & Guimaraens, 1994; Conde-Salazar, Guimaraens, Villegas, Romero, & Gonzalez, 1995; Estlander, Jolanki, Henriks-Eckerman, & Kanerva, 1999; Gardiner, Waechter, Wiedow, & Solomon, 1992; Goulden & Wilkinson, 1996; Holmes et al., 1993; Jolanki et al., 1996; Kanerva et al., 1991; Kiec-Swierczynska, 1995; Le Coz et al., 1999; Rempel et al., 1991; Wang, Lin, Chen, & Ye, 1992). Typically, the epidemiologist is able to define the time period of exposure, which helps define the cause of the health effect, but this does not fully explain a dose response relationship. One attempt to quantify worker dose would be to use a surrogate for exposure, such as pounds of epoxy used. Through this method, a more complete picture could be developed in order to create a better model of the relationship between health effect and exposure.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The first goal of this project was to determine if ion chromatography sufficient analytical tool for measuring ambient concentrations of epoxy resins using a method specified by Dr. Robert Herrick (Herrick & Smith, 1987). Two different purities of digylcidyl ether of bisphenol A (DGBA), a model epoxy compound, were analyzed to determine recovery rates using four standards at each of five different loading levels. Results obtained by Dr. Herrick using normal pulse polarography were then compared to the results obtained using ion chromatography in order to assess the adequacy of the method.

The second goal of the project was to determine the stability of the reactants to see if the method outlined by Dr. Herrick is applicable to field industrial hygiene sampling. All forty standards used in part one of this project were analyzed at periodic intervals following the initial reaction procedure to determine the amount of change in recovery rate over time.

In order to evaluate if ion chromatography was an appropriate analytical tool for this method, the mean, standard deviation, and coefficients of variation were calculated for each of the five different loading levels for both purities of DGBA for each of the days that the standards were analyzed. The results were compared to the requirements set forth by the National Institute for Occupational Health and Safety (NIOSH) which was a

75 percent recovery rate 95 percent of the time (National Institute for Occupational Safety and Health, 1994). For both purities of DGBA, this requirement was met for the two highest loading levels, but not for the other three. In addition, the minimum level repeatably recovered as well as coefficients of variation were compared with results obtained by Dr. Herrick using normal pulse polarography. Dr. Herrick was able to repeatably detect 0.17 mg of DGBA with a coefficient of variation of 0.06. Using ion chromatography, the minimum repeatably detectable level was 107 mg of DGBA with a coefficient of variation of 2.85. The differences in results are likely due to overloading of the column, resulting in standard contamination from one standard to another. This contributed to both poor recovery rates, especially at the lower loading levels, as well as significant variability between standards analyzed after blank analyses and those analyzed after other standards.

Assessment of standard stability was accomplished by comparing recovery rates for each of the loading levels analyzed on day 0 and the day of interest. Multiple pair wise comparisons were performed to determine significant differences between each of the groups. Only the two highest loading levels had small enough variation in data to be able to determine significant differences between the initial analysis and the analysis of interest. Subsequently, regression models were developed in order to determine the maximum time that the standards would still be viable using the NIOSH requirements. For each of the purities of DGBA at the two highest loading levels, the maximum time the standards remained viable were determined to be approximately 20 days. This demonstrates that this method is applicable to industrial hygiene field operations since the standards would be considered stable for approximately three weeks.

Overall, superficial analysis of the results from this project would indicate that ion chromatography is not an adequate analytical tool for determining ambient levels of epoxy resins. However, since carryover appears to be the primary cause of the poor recovery rates, especially at the lower loading levels, elimination of carryover should result in significantly improved results. Once this is accomplished, this and other future experiments could be performed in order to demonstrate the effectiveness of ion chromatography as an analytical tool for determining epoxy resin concentrations.

CHAPTER VII

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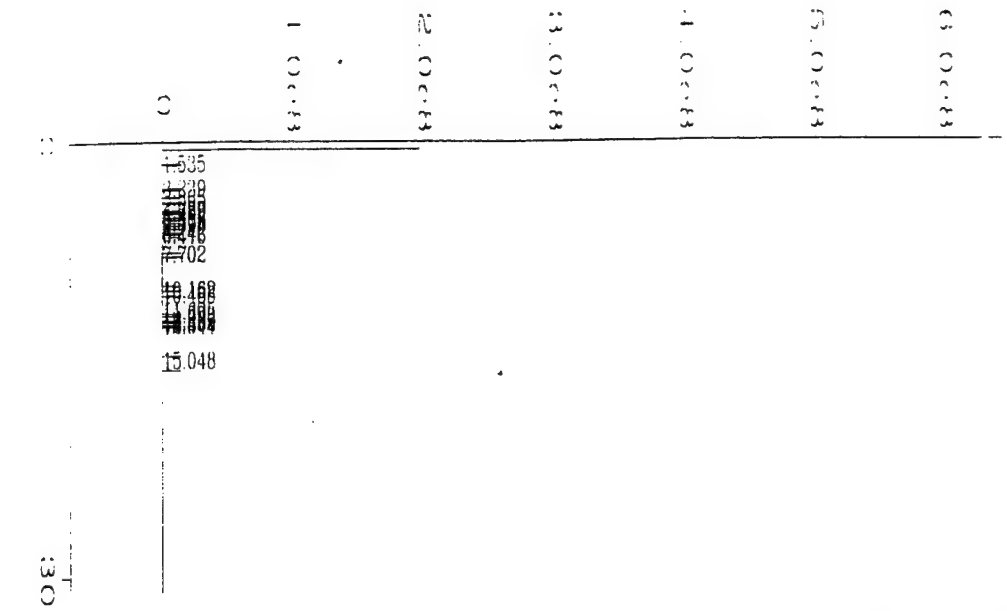
APPENDICES

Appendix I: Abbreviations

AAS	Atomic Absorption Spectrometry
ALT	Aminotransferase
AST	Aspartate aminotransferase
ASTM	American Society of Testing and Materials
cm	Centimeter
DGBA	Diglycidyl Ether of Bisphenol A
DMF	Dimethylformamide
EPA	Environmental Protection Agency
g	Grams
IARC	International Agency for Research on Cancer
i.d.	Internal Diameter
kPa	KiloPascals
mL	Milliliter
M	Molar
mM	Millimolar
mmHg	Millimeters of Mercury
N	Normal
NIOSH	National Institute of Occupational Safety and Health
NPP	Normal Pulse Polarography
NWRC	National Wildlife Research Center
Pa	Pascals
ppm	Parts Per Million

TEAB	Tetraethylammonium bromide
TLV	Threshold Limit Value
uL	Microliters
um	Micron
uS	Microsiemens
VHI	Vapor Hazard Index
VP	Vapor Pressure

Appendix II: Gas Chromatographs used to determine DGBA purity



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Area Percent Report

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Instrument : INSTRUMEN
Sample Name : Precipitated
Run Time Bar Code:
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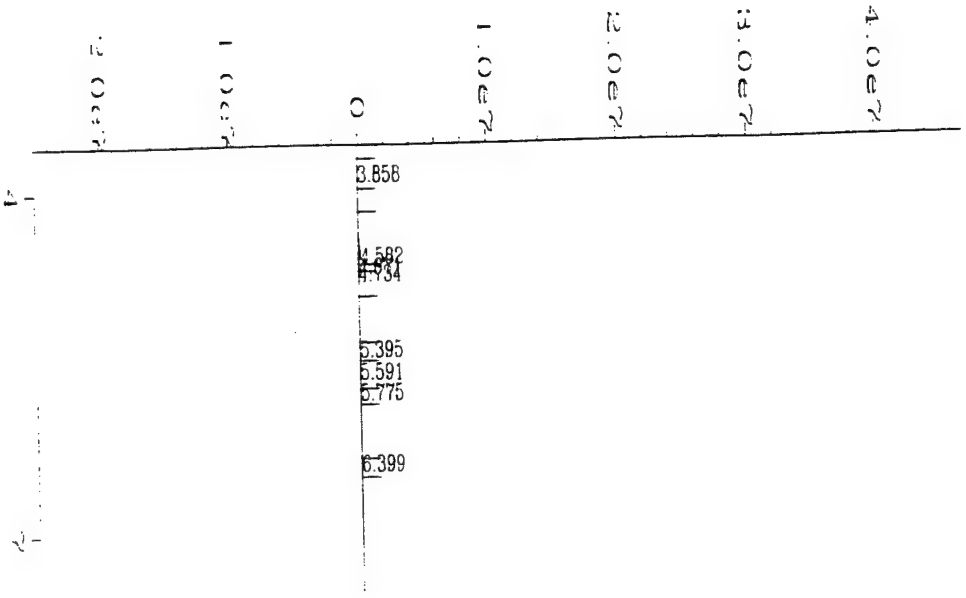
Report Created on: 26 Jan 00 02:10 PM

Page Number : 1
Vial Number : 1
Injection Number : 5
Sequence Line : 2
Instrument Method: BISPHEN.MTH
Analysis Method : BISPHEN.MTH

Sig. 1 in C:\HPCHEM\1\DATA\JAY\00011906.D

Pk#	Ret Time	Area	Height	Type	Width	Area %
1	1.535	131	136	BV	0.016	0.0067
2	3.239	924	436	BV	0.052	0.0474
3	3.865	1050	154	BB	0.102	0.0538
4	4.659	1830564	184132	BV	0.131	93.8871
5	4.725	1117	605	VV	0.031	0.0573
6	4.780	897	345	VV	0.046	0.0460
7	5.238	481	304	PV	0.026	0.0247
8	5.416	3030	1328	PV	0.039	0.1554
9	5.598	2357	1123	PV	0.050	0.1209
10	5.794	901	451	VV	0.044	0.0462
11	6.242	352	177	BV	0.031	0.0180
12	6.416	1048	556	PV	0.030	0.0537
13	7.702	7208	3737	BB	0.050	0.3697
14	10.162	237	58	PV	0.062	0.0121
15	10.488	1133	221	VV	0.077	0.0581
16	11.625	15095	320	VV	0.573	0.7742
17	11.893	1839	189	VV	0.148	0.0943
18	12.202	2698	137	VV	0.238	0.1384
19	12.369	658	84	VV	0.123	0.0338
20	12.462	609	74	VV	0.136	0.0312



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Area Percent Report

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Report Created on: 26 Jan 00 01:59 PM

Page Number : 1
Vial Number : 2
Injection Number : 5
Sequence Line : 2
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Analysis Method : BISPHEN.MTH

Sig. 1 in C:\HPCHEM\1\DATA\JAY\00011911.D

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3	4.671	1453	932	VV	0.026	0.1163
4	4.734	1077	453	VB	0.045	0.0862
5	5.395	7480	3281	BV	0.039	0.5988
6	5.591	13534	6783	VV	0.031	1.0835
7	5.775	2800	1611	VV	0.042	0.2242
8	6.399	914	482	BB	0.044	0.0732
9	7.690	8394	4171	BB	0.032	0.6720
10	9.404	175	272	PV	0.011	0.0140
11	9.770	103	21	PV	0.081	0.0082
12	9.996	167	40	PV	0.063	0.0133
13	10.484	1808	328	VV	0.071	0.1447
14	11.618	16661	429	VV	0.471	1.3338
15	11.802	1596	251	VV	0.092	0.1278
16	11.893	1440	242	VV	0.078	0.1152
17	12.198	3243	210	VV	0.195	0.2596
18	12.454	2597	163	VV	0.196	0.2079
19	12.624	555	110	VV	0.084	0.0445
20	12.927	1533	118	VB	0.168	0.1227

Appendix III: Individual Ion Chromatography Standard Results

Table 10 – Individual ion chromatography standard recovery rates in percent. (P) represents the precipitated product (94 percent pure) and (TCI) represents the original 87 percent pure product

Sample #		Loading Level	Day 0	Day 1	Day 7	Day 14	Day 28	Day 29
P	1	0.94	-1198.99	-1246.59	-1379.04	-2281.89	585.43	534.47
P	2	3.76	-179.73	-201.89	-65.80	-4.63	-18.88	142.46
P	3	37.56	104.66	102.02	90.88	76.74	61.00	36.18
P	4	112.68	111.76	106.96	102.07	88.14	65.55	61.28
P	5	187.80	100.02	99.15	93.82	86.84	65.07	67.50
P	6	0.94	-3162.14	-3036.59	10237.77	-2972.38	-2231.92	-1192.04
P	7	3.76	-575.10	-870.48	-675.50	-386.16	-374.55	-155.89
P	8	37.56	42.48	300.55	56.83	45.88	55.09	68.24
P	9	112.68	103.32	102.05	94.58	83.36	62.81	66.57
P	10	187.80	101.08	99.51	94.16	85.20	68.24	66.09
P	11	0.94	-1661.08	-1350.19	-1384.05	-528.82	-42.96	1316.54
P	12	3.76	-461.53	-357.09	-156.68	-148.50	74.51	144.90
P	13	37.56	91.00	91.13	75.51	96.62	80.78	59.85
P	14	112.68	112.04	112.21	103.53	103.63	78.39	65.08
P	15	187.80	101.42	99.93	94.57	87.48	70.28	68.50
P	16	0.94	-3038.35	-2841.81	-3297.01	-2854.53	-827.15	-1489.88
P	17	3.76	2999.561	-858.60	-741.34	-418.61	-268.57	-343.47
P	18	37.56	35.05	90.00	51.12	57.77	56.30	56.19
P	19	112.68	104.30	98.65	93.95	80.97	66.42	64.95
P	20	187.80	96.71	96.35	91.83	81.11	66.17	56.00
TCI	1	0.89	-1603.48	-694.44	552.65	1051.96	1940.54	2526.18
TCI	2	3.58	-289.20	-325.74	-192.88	-12.75	141.92	249.10
TCI	3	35.76	82.68	97.61	77.02	110.58	98.06	62.18
TCI	4	107.28	108.51	106.95	96.50	89.50	72.87	60.98
TCI	5	178.80	99.31	96.42	92.82	85.99	71.94	64.90
TCI	6	0.89	-3432.85	-3530.70	-3576.79	-2505.85	-1376.08	-2003.32
TCI	7	3.58	-1089.67	-861.30	-730.81	-568.00	-317.68	-202.48
TCI	8	35.76	27.12	50.33	41.88	31.65	41.09	434.83
TCI	9	107.28	103.53	100.01	93.03	78.46	67.98	65.75
TCI	10	178.80	101.35	100.88	96.28	86.84	72.57	71.20
TCI	11	0.89	-1870.32	-2228.07	-2055.84	-967.35	-245.12	926.56
TCI	12	3.58	-641.42	-287.51	-210.68	-243.85	136.08	214.26
TCI	13	35.76	82.46	83.70	79.54	94.06	89.16	58.75
TCI	14	107.28	110.14	109.43	98.94	90.75	66.14	62.38
TCI	15	178.80	103.76	101.37	96.02	87.27	71.97	67.01
TCI	16	0.89	-2869.57	-3386.13	-3834.55	-2958.02	-2611.84	-1889.74
TCI	17	3.58	-1129.13	-988.22	-856.71	-609.01	-446.52	-237.60
TCI	18	35.76	36.65	51.82	28.74	59.58	26.13	38.32
TCI	19	107.28	105.06	98.65	90.80	85.76	67.99	62.56
TCI	20	178.80	101.76	99.33	94.25	83.80	69.24	66.56

Appendix IV: Multiple pair wise comparisons of standards analyzed after standards and blanks were analyzed (group 1), and those that were not analyzed after standards and blanks were analyzed (group 2)

Precipitated DGBA

Analysis of Variance for Rec0.94

Source	DF	SS	MS	F	P
Group	1	17170135	17170135	17.41	0.000
Error	21	20714910	986424		
Total	22	37885045			

Level	N	Mean	StDev
1	12	-719.8	1088.1
2	11	-2449.4	876.9

Pooled StDev = 993.2

Individual 95% CIs For Mean Based on Pooled StDev

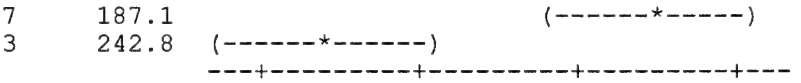
Analysis of Variance for Loading Level 3.8

Source	DF	SS	MS	F	P
Group	1	976828	976828	21.04	0.000
Error	21	974830	46420		
Total	22	1951658			

Level	N	Mean	StDev
1	12	-102.7	187.1
2	11	-515.3	242.8

Pooled StDev = 215.5

Individual 95% CIs For Mean Based on Pooled StDev



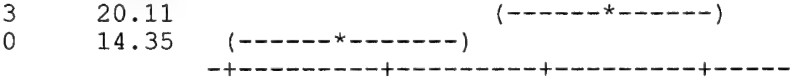
Analysis of Variance for Loading Level 38

Source	DF	SS	MS	F	P
Group	1	3481	3481	11.23	0.003
Error	21	6506	310		
Total	22	9987			

Level	N	Mean	StDev
1	12	80.53	20.11
2	11	55.90	14.35

Pooled StDev = 17.60

Individual 95% CIs For Mean Based on Pooled StDev



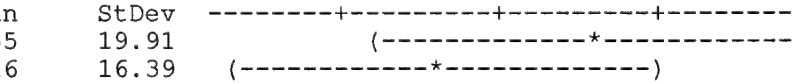
Analysis of Variance for Loading Level 113

Source	DF	SS	MS	F	P
Group	1	328	328	0.99	0.331
Error	22	7313	332		
Total	23	7641			

Level	N	Mean	StDev
1	12	92.55	19.91
2	12	85.16	16.39

Pooled StDev = 18.23

Individual 95% CIs For Mean Based on Pooled StDev

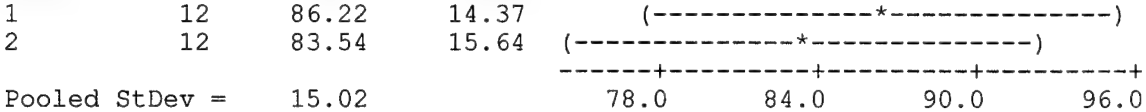


Analysis of Variance for Loading Level 188

Source	DF	SS	MS	F	P
Group	1	43	43	0.19	0.667
Error	22	4961	226		
Total	23	5004			

Level	N	Mean	StDev
1	12	86.22	14.37
2	12	83.54	15.64
Pooled StDev =		15.02	

Individual 95% CIs For Mean Based on Pooled StDev



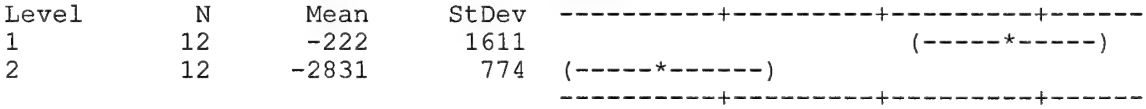
Unpure Product Comparisons

Analysis of Variance for Loading Level 0.89

Source	DF	SS	MS	F	P
Group	1	40843138	40843138	25.57	0.000
Error	22	35145811	1597537		
Total	23	75988949			

Level	N	Mean	StDev
1	12	-222	1611
2	12	-2831	774
Pooled StDev =		1264	

Individual 95% CIs For Mean Based on Pooled StDev

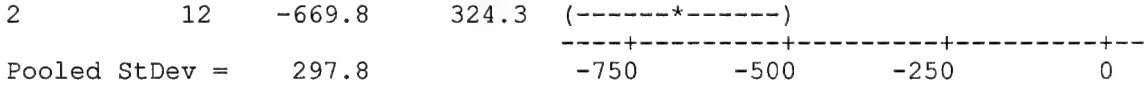


Analysis of Variance for Loading Level 3.6

Source	DF	SS	MS	F	P
Group	1	1800980	1800980	20.31	0.000
Error	22	1950454	88657		
Total	23	3751435			

Level	N	Mean	StDev
1	12	-121.9	268.6
2	12	-669.8	324.3
Pooled StDev =		297.8	

Individual 95% CIs For Mean Based on Pooled StDev

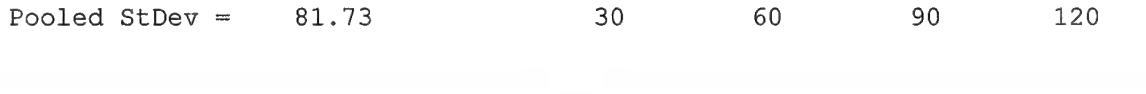


Analysis of Variance for Loading Level 36

Source	DF	SS	MS	F	P
Group	1	908	908	0.14	0.716
Error	22	146938	6679		
Total	23	147847			

Level	N	Mean	StDev
1	12	84.65	14.77
2	12	72.35	114.63
Pooled StDev =		81.73	

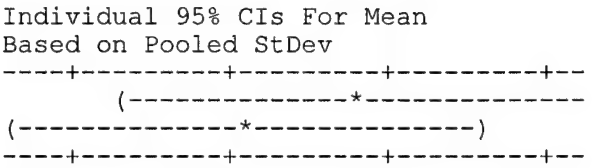
Individual 95% CIs For Mean Based on Pooled StDev



Analysis of Variance for Loading Level 107

Source	DF	SS	MS	F	P
Group	1	119	119	0.39	0.539
Error	22	6752	307		
Total	23	6871			

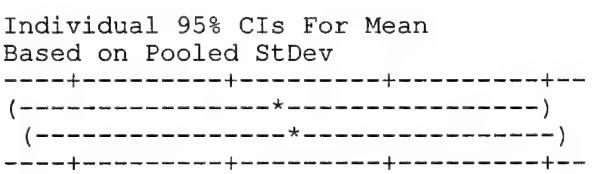
Level	N	Mean	StDev
1	12	89.42	19.07
2	12	84.97	15.82
Pooled StDev =		17.52	



Analysis of Variance for Loading Level 179

Source	DF	SS	MS	F	P
Group	1	1	1	0.01	0.939
Error	22	4291	195		
Total	23	4292			

Level	N	Mean	StDev
1	12	86.57	14.09
2	12	87.01	13.84
Pooled StDev =		13.97	



Appendix V: Multiple pair wise comparisons of different standard groups

Precipitated Product

Concentration (mg)	Comparison Day	Statistically Different
1	1	No
1	7	No
1	14	No
1	28	No
1	29	No
4	1	No
4	7	No
4	14	No
4	28	No
4	29	No
40	1	No
40	7	No
40	14	No
40	28	No
40	29	No
120	1	No
120	7	No
120	**14**	**Yes**
120	**28**	**Yes**
120	**29**	**Yes**
200	1	No
200	**7**	**Yes**
200	**14**	**Yes**
200	**28**	**Yes**
200	**29**	**Yes**

TCI Product

Concentration (mg)	Comparison Day	Statistically Different
0.89	1	No
0.89	7	No
0.89	14	No
0.89	28	No
0.89	29	No
3.6	1	No
3.6	7	No
3.6	14	No
3.6	28	No
3.6	**29**	**Yes**
36	1	No
36	7	No
36	14	No
36	28	No
36	29	No
107	1	No
107	**7**	**Yes**
107	**14**	**Yes**
107	**28**	**Yes**
107	**29**	**Yes**
179	1	No
179	**7**	**Yes**
179	**14**	**Yes**
179	**28**	**Yes**
179	**29**	**Yes**

Appendix VI: Analysis of variance and regression modeling with precipitated product

ANOVA's conducted with removal of P-6(7), P-8(1) and P-17(0)

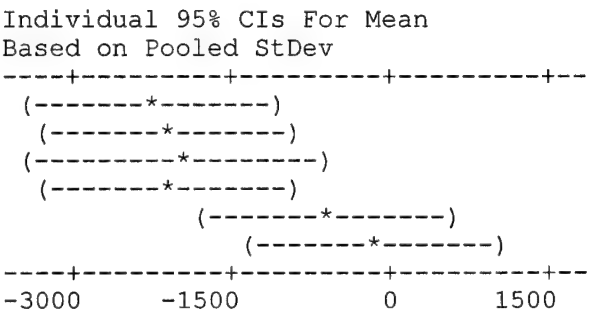
Analysis of Variance for Loading Level 0.94

Source	DF	SS	MS	F	P
Day	5	16086537	3217307	2.51	0.071
Error	17	21798508	1282265		
Total	22	37885045			

Level	N	Mean	StDev
0	4	-2265	984
1	4	-2119	952
7	3	-2020	1106
14	4	-2159	1128
28	4	-629	1215
29	4	-208	1352

Pooled StDev = 1132

Individual 95% CIs For Mean Based on Pooled StDev



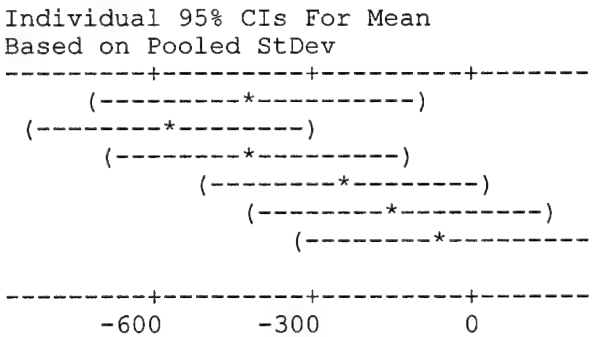
Analysis of Variance for Loading Level 3.8

Source	DF	SS	MS	F	P
Day	5	730062	146012	2.03	0.125
Error	17	1221596	71859		
Total	22	1951658			

Level	N	Mean	StDev
0	3	-405.5	203.6
1	4	-572.0	343.7
7	4	-409.8	347.8
14	4	-239.5	197.5
28	4	-146.9	209.8
29	4	-53.0	239.7

Pooled StDev = 268.1

Individual 95% CIs For Mean Based on Pooled StDev



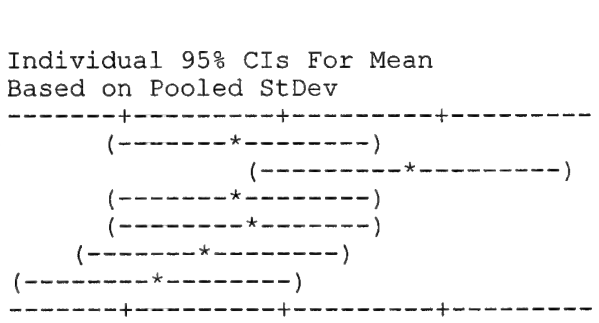
Analysis of Variance for Loading Level 38

Source	DF	SS	MS	F	P
Day	5	2836	567	1.35	0.292
Error	17	7151	421		
Total	22	9987			

Level	N	Mean	StDev
0	4	68.30	34.69
1	3	94.38	6.64
7	4	68.58	18.15
14	4	69.25	22.23
28	4	63.29	11.93
29	4	55.11	13.59

Pooled StDev = 20.51

Individual 95% CIs For Mean Based on Pooled StDev



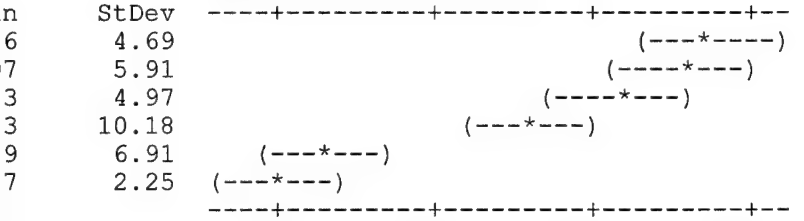
Analysis of Variance for Loading Level 113

Source	DF	SS	MS	F	P
Day	5	6927.0	1385.4	34.91	0.000
Error	18	714.2	39.7		
Total	23	7641.2			

Level	N	Mean	StDev
0	4	107.86	4.69
1	4	104.97	5.91
7	4	98.53	4.97
14	4	89.03	10.18
28	4	68.29	6.91
29	4	64.47	2.25

Pooled StDev = 6.30

Individual 95% CIs For Mean Based on Pooled StDev



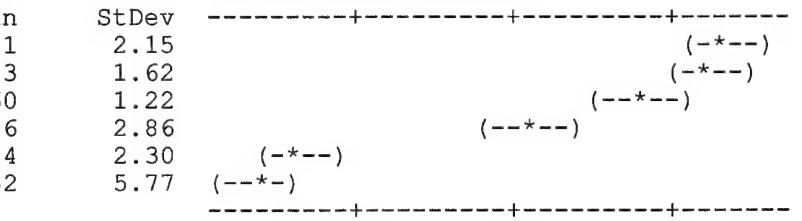
Analysis of Variance for Loading Level 188

Source	DF	SS	MS	F	P
Day	5	4837.60	967.52	104.59	0.000
Error	18	166.50	9.25		
Total	23	5004.11			

Level	N	Mean	StDev
0	4	99.81	2.15
1	4	98.73	1.62
7	4	93.60	1.22
14	4	85.16	2.86
28	4	67.44	2.30
29	4	64.52	5.77

Pooled StDev = 3.04

Individual 95% CIs For Mean Based on Pooled StDev



Regression Analysis for Loading Level 188

The regression equation is
Rec188 = 101 - 1.20 Day

Predictor	Coef	StDev	T	P
Constant	100.718	0.907	110.99	0.000
Day	-1.20319	0.05139	-23.41	0.000

S = 2.962 R-Sq = 96.1% R-Sq(adj) = 96.0%

Confidence and Predictive Intervals for Loading Level 188

Fit	StDev Fit	95.0% CI		95.0% PI		Day
82.670	0.612	(81.401,	83.940)	(76.396,	88.945)	15
81.467	0.622	(80.177,	82.757)	(75.188,	87.746)	16
80.264	0.636	(78.945,	81.583)	(73.979,	86.549)	17
79.061	0.654	(77.705,	80.417)	(72.768,	85.354)	18
77.858	0.675	(76.458,	79.258)	(71.555,	84.160)	19
76.654	0.699	(75.204,	78.105)	(70.340,	82.969)	20
75.451	0.726	(73.944,	76.958)	(69.124,	81.779)	21
74.248	0.756	(72.680,	75.817)	(67.906,	80.590)	22
73.045	0.788	(71.410,	74.680)	(66.686,	79.404)	23
71.842	0.822	(70.137,	73.547)	(65.464,	78.219)	24
70.639	0.858	(68.860,	72.417)	(64.241,	77.036)	25

Regression Analysis for Loading Level 113

The regression equation is
Rec113 = 108 - 1.44 Day

Predictor	Coef	StDev	T	P
Constant	107.824	1.782	60.50	0.000
Day	-1.4405	0.1009	-14.27	0.000

S = 5.818 R-Sq = 90.3% R-Sq(adj) = 89.8%

Confidence and Predictive Intervals for Loading Level 113

Fit	StDev Fit	95.0% CI		95.0% PI		Day
86.22	1.20	(83.72,	88.71)	(73.89,	98.54)	15
84.78	1.22	(82.24,	87.31)	(72.44,	97.11)	16
83.34	1.25	(80.74,	85.93)	(70.99,	95.68)	17
81.89	1.28	(79.23,	84.56)	(69.53,	94.25)	18
80.45	1.33	(77.70,	83.20)	(68.08,	92.83)	19
79.01	1.37	(76.16,	81.86)	(66.61,	91.41)	20
77.57	1.43	(74.61,	80.53)	(65.15,	90.00)	21
76.13	1.49	(73.05,	79.21)	(63.68,	88.59)	22
74.69	1.55	(71.48,	77.90)	(62.20,	87.18)	23
73.25	1.61	(69.90,	76.60)	(60.73,	85.78)	24
71.81	1.68	(68.32,	75.30)	(59.25,	84.38)	25

Regression Analysis for Loading Level 0.94

The regression equation is
Rec0.94 = - 2413 + 64.5 Day

Predictor	Coef	StDev	T	P
Constant	-2412.9	337.1	-7.16	0.000
Day	64.45	18.75	3.44	0.002

S = 1074 R-Sq = 36.0% R-Sq(adj) = 33.0%

Regression Analysis for Loading Level 3.8

The regression equation is
Rec3.8 = - 497 + 14.3 Day

Predictor	Coef	StDev	T	P
Constant	-496.99	80.25	-6.19	0.000
Day	14.334	4.449	3.22	0.004

S = 249.4 R-Sq = 33.1% R-Sq(adj) = 29.9%

Regression Analysis for Loading Level 38

The regression equation is
Rec38 = 77.8 - 0.661 Day

Predictor	Coef	StDev	T	P
Constant	77.801	6.486	12.00	0.000
Day	-0.6606	0.3596	-1.84	0.080

S = 20.24 R-Sq = 13.8% R-Sq(adj) = 9.7%

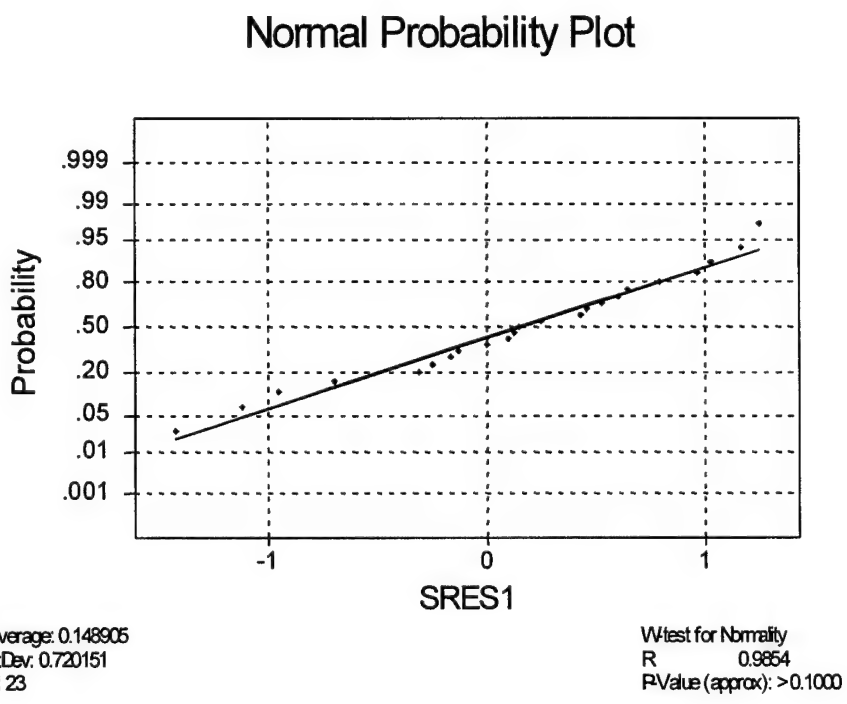


Figure 9 – Normality Test for loading level 188 using standardized residuals of one-way ANOVA

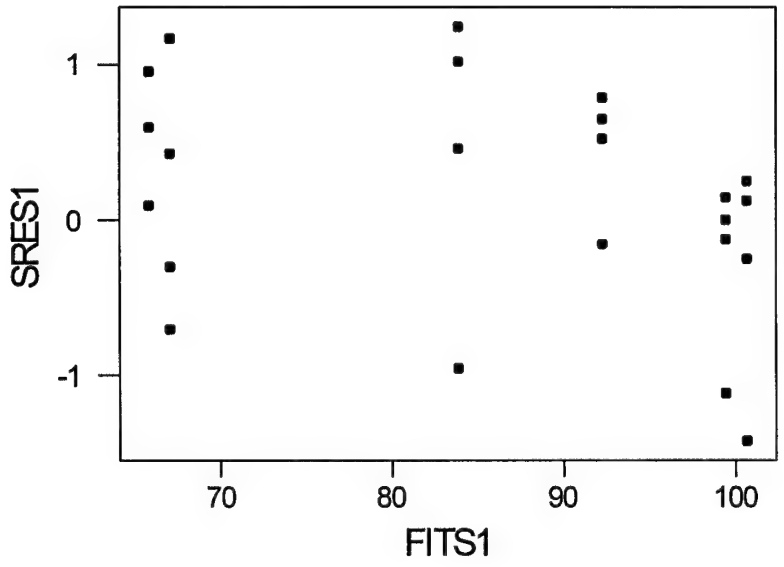


Figure 10 – Test for homogeneity of variance for loading level 188 using standardized residuals versus fitted values of one-way ANOVA

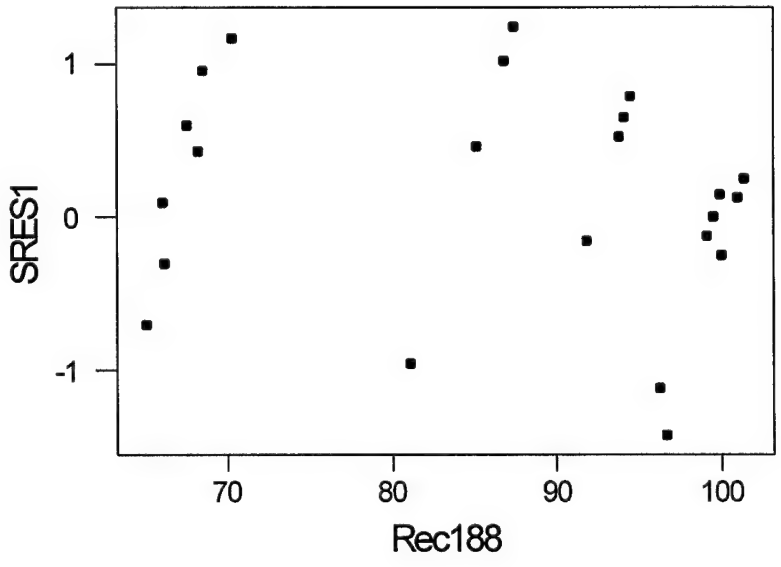


Figure 11 -- Test for linearity for loading level 188 using standardized residuals versus recovery rate of one-way ANOVA

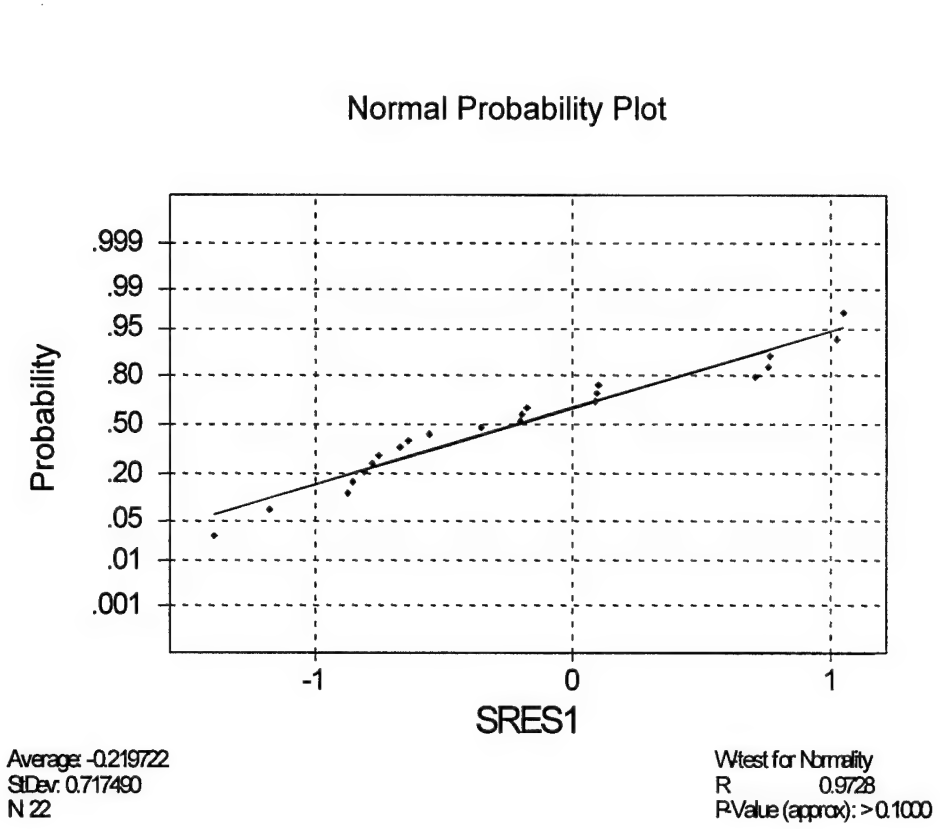


Figure 12 -- Normality Test for loading level 113 using standardized residuals of one-way ANOVA

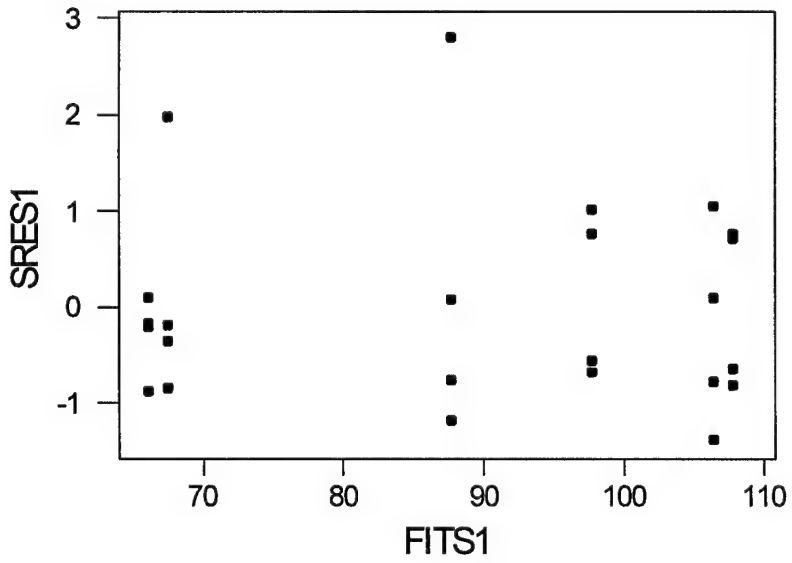


Figure 13 -- Test for homogeneity of variance for loading level 113 using standardized residuals versus fitted values of one-way ANOVA

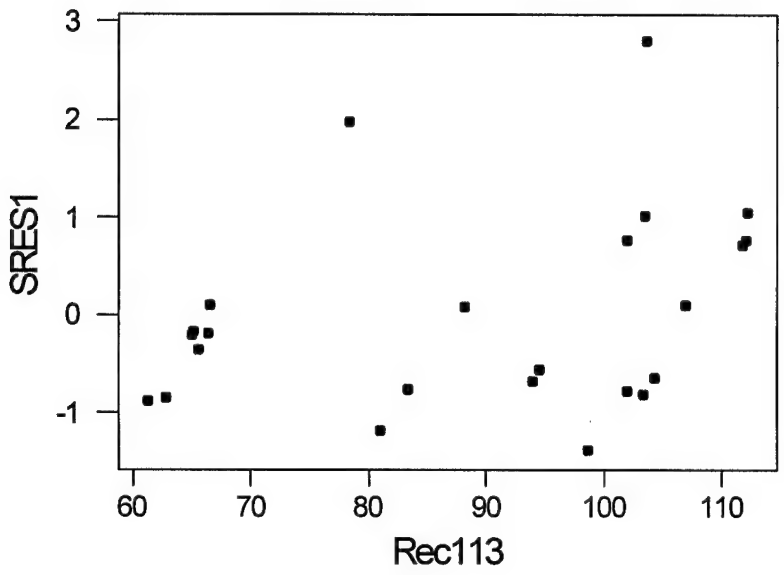


Figure 14 -- Test for linearity for loading level 113 using standardized residuals versus recovery rate of one-way ANOVA

Appendix VII: Analysis of variance and regression modeling with TCI America DGBA product

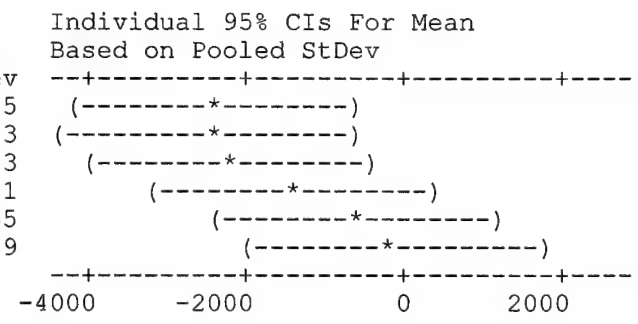
Analysis of Variance for Loading Level 0.89

Source	DF	SS	MS	F	P
Day	5	20616760	4123352	1.34	0.292
Error	18	55372189	3076233		
Total	23	75988949			

Level	N	Mean	StDev
0	4	-2444	855
1	4	-2460	1313
7	4	-2229	2013
14	4	-1345	1811
28	4	-573	1935
29	4	-110	2219

Pooled StDev = 1754

Individual 95% CIs For Mean Based on Pooled StDev



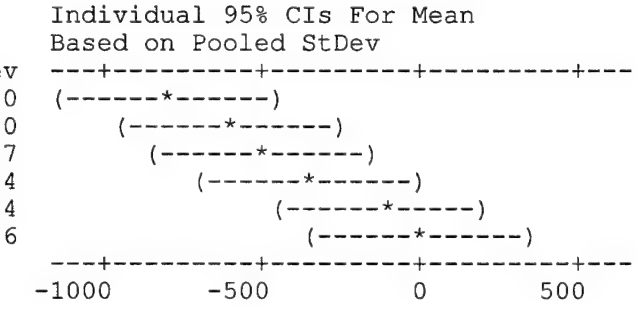
Analysis of Variance for Loading Level 3.6

Source	DF	SS	MS	F	P
Day	5	1799905	359981	3.32	0.027
Error	18	1951530	108418		
Total	23	3751435			

Level	N	Mean	StDev
0	4	-787.4	399.0
1	4	-615.7	361.0
7	4	-497.8	345.7
14	4	-358.4	282.4
28	4	-121.6	305.4
29	4	5.8	261.6

Pooled StDev = 329.3

Individual 95% CIs For Mean Based on Pooled StDev



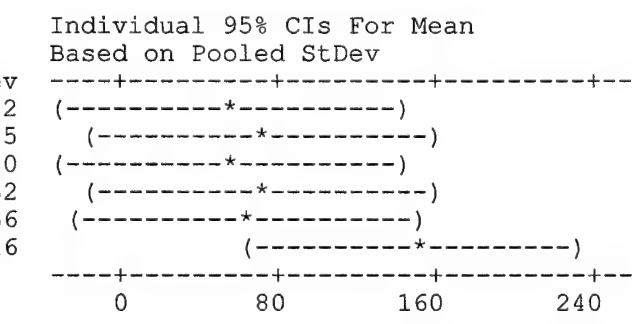
Analysis of Variance for Loading Level 36

Source	DF	SS	MS	F	P
Day	5	24508	4902	0.72	0.620
Error	18	123339	6852		
Total	23	147847			

Level	N	Mean	StDev
0	4	57.23	29.52
1	4	70.86	23.55
7	4	56.80	25.40
14	4	73.97	35.32
28	4	63.61	35.36
29	4	148.52	191.16

Pooled StDev = 82.78

Individual 95% CIs For Mean Based on Pooled StDev



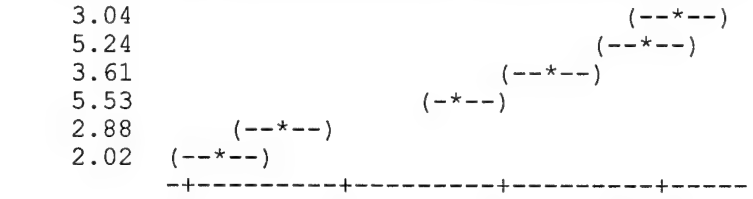
Analysis of Variance for Loading Level 107

Source	DF	SS	MS	F	P
Day	5	6592.8	1318.6	85.29	0.000
Error	18	278.3	15.5		
Total	23	6871.1			

Level	N	Mean	StDev
0	4	106.81	3.04
1	4	103.76	5.24
7	4	94.82	3.61
14	4	86.12	5.53
28	4	68.75	2.88
29	4	62.92	2.02

Pooled StDev = 3.93

Individual 95% CIs For Mean Based on Pooled StDev



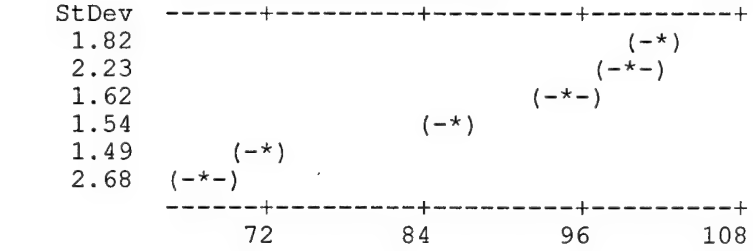
Analysis of Variance for Loading Level 179

Source	DF	SS	MS	F	P
Day	5	4223.94	844.79	223.14	0.000
Error	18	68.15	3.79		
Total	23	4292.08			

Level	N	Mean	StDev
0	4	101.55	1.82
1	4	99.50	2.23
7	4	94.84	1.62
14	4	85.98	1.54
28	4	71.43	1.49
29	4	67.42	2.68

Pooled StDev = 1.95

Individual 95% CIs For Mean Based on Pooled StDev



Regression Analysis for Loading Level 179

The regression equation is
Rec179 = 102 - 1.12 Day

Predictor	Coef	StDev	T	P
Constant	101.584	0.632	160.61	0.000
Day	-1.12396	0.03582	-31.38	0.000

S = 2.065 R-Sq = 97.8% R-Sq(adj) = 97.7%

Confidence and Predictive Intervals for Loading Level 179

Fit	StDev Fit	95.0% CI	95.0% PI	Days
84.724	0.427	(83.840, 85.609)	(80.351, 89.098)	15
83.600	0.434	(82.701, 84.500)	(79.224, 87.977)	16
82.477	0.443	(81.557, 83.396)	(78.096, 86.857)	17
81.353	0.456	(80.407, 82.298)	(76.966, 85.739)	18
80.229	0.470	(79.253, 81.204)	(75.836, 84.621)	19
79.105	0.487	(78.094, 80.116)	(74.704, 83.505)	20
77.981	0.506	(76.930, 79.031)	(73.571, 82.391)	21
76.857	0.527	(75.764, 77.950)	(72.436, 81.277)	22
75.733	0.549	(74.593, 76.872)	(71.301, 80.165)	23
74.609	0.573	(73.420, 75.797)	(70.164, 79.054)	24
73.485	0.598	(72.245, 74.725)	(69.026, 77.944)	25

Regression Analysis for Loading Level 107

The regression equation is
Rec107 = 106 - 1.40 Day

Predictor	Coef	StDev	T	P
Constant	105.669	1.183	89.32	0.000
Day	-1.40314	0.06699	-20.95	0.000

S = 3.862 R-Sq = 95.2% R-Sq(adj) = 95.0%

Confidence and Predictive Intervals for Loading Level 107

Fit	StDev Fit	95.0% CI	95.0% PI	Days
84.622	0.798	(82.967, 86.277)	(76.442, 92.802)	15
83.219	0.811	(81.537, 84.901)	(75.033, 91.405)	16
81.816	0.829	(80.096, 83.536)	(73.622, 90.009)	17
80.413	0.852	(78.645, 82.181)	(72.209, 88.617)	18
79.010	0.880	(77.184, 80.835)	(70.793, 87.226)	19
77.606	0.912	(75.715, 79.497)	(69.375, 85.838)	20
76.203	0.947	(74.239, 78.168)	(67.955, 84.452)	21
74.800	0.986	(72.755, 76.845)	(66.532, 83.068)	22
73.397	1.027	(71.266, 75.528)	(65.107, 81.687)	23
71.994	1.072	(69.771, 74.217)	(63.680, 80.308)	24
70.591	1.118	(68.272, 72.910)	(62.251, 78.931)	25

Regression Analysis for Loading Level 0.89

The regression equation is
Rec0.89 = - 2549 + 77.6 Day

Predictor	Coef	StDev	T	P
Constant	-2548.7	488.6	-5.22	0.000
Day	77.62	27.67	2.81	0.010

S = 1595 R-Sq = 26.3% R-Sq(adj) = 23.0%

Regression Analysis for Loading Level 3.6

The regression equation is
Rec3.6 = - 696 + 22.8 Day

Predictor	Coef	StDev	T	P
Constant	-695.63	93.01	-7.48	0.000
Day	22.770	5.267	4.32	0.000

S = 303.6 R-Sq = 45.9% R-Sq(adj) = 43.5%

Regression Analysis for Loading Level 36

The regression equation is
Rec36 = 56.5 + 1.67 Day

Predictor	Coef	StDev	T	P
Constant	56.46	24.31	2.32	0.030
Day	1.674	1.377	1.22	0.237

S = 79.35 R-Sq = 6.3% R-Sq(adj) = 2.0%

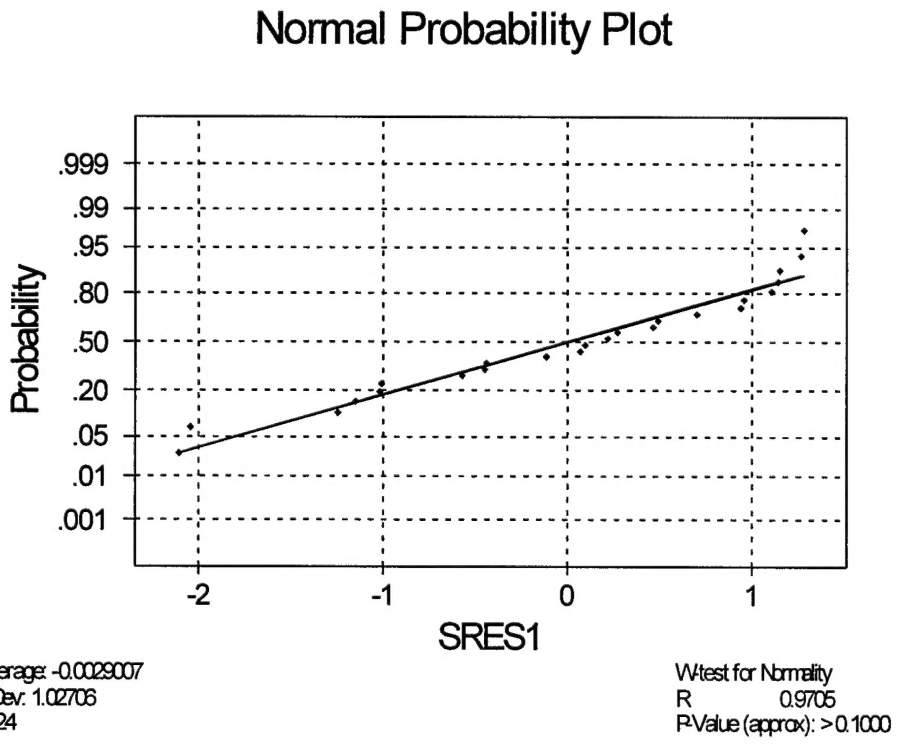


Figure 15 -- Normality Test for loading level 179 using standardized residuals of one-way ANOVA

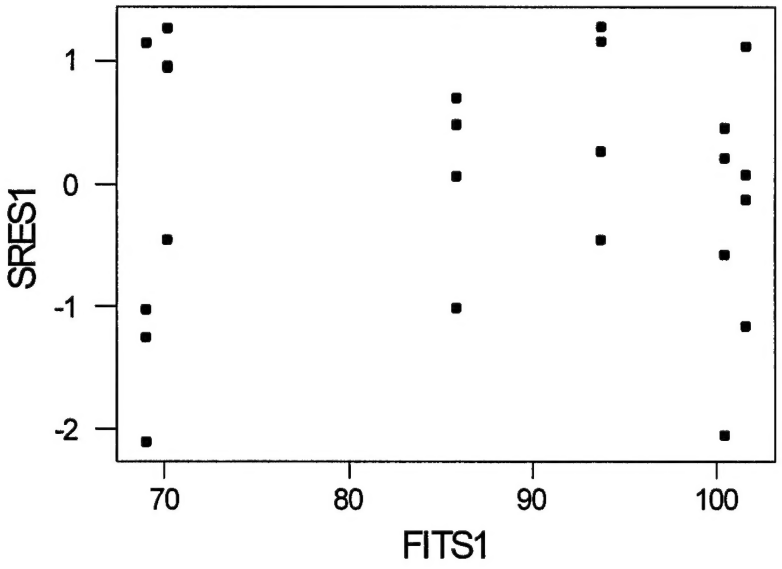


Figure 16 -- Test for homogeneity of variance for loading level 179 using standardized residuals versus fitted values of one-way ANOVA

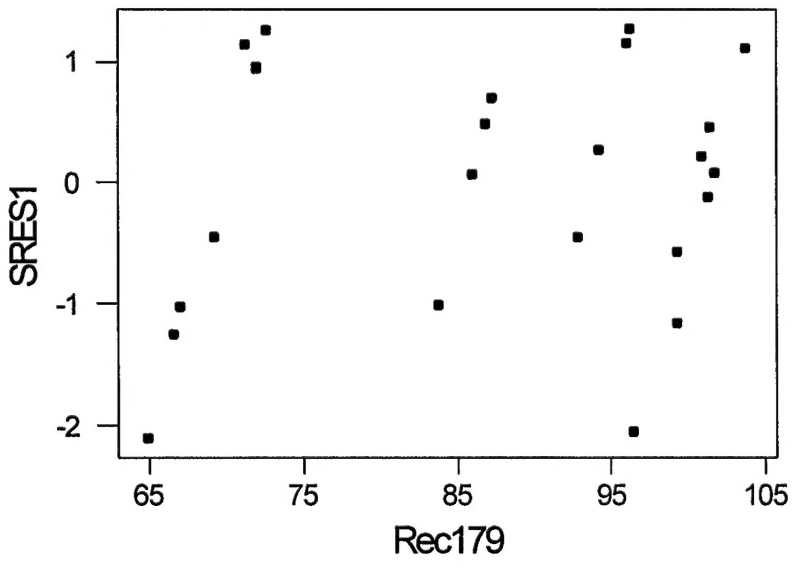


Figure 17-- Test for linearity for loading level 179 using standardized residuals versus recovery rate of one-way ANOVA

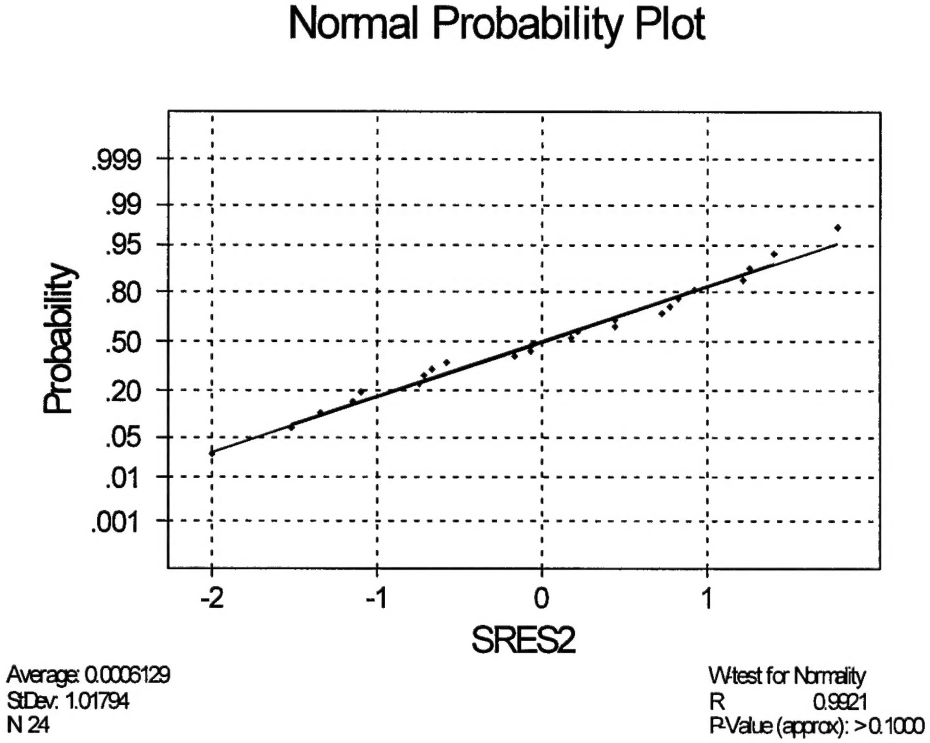


Figure 18 -- Normality Test for loading level 107 using standardized residuals of one-way ANOVA

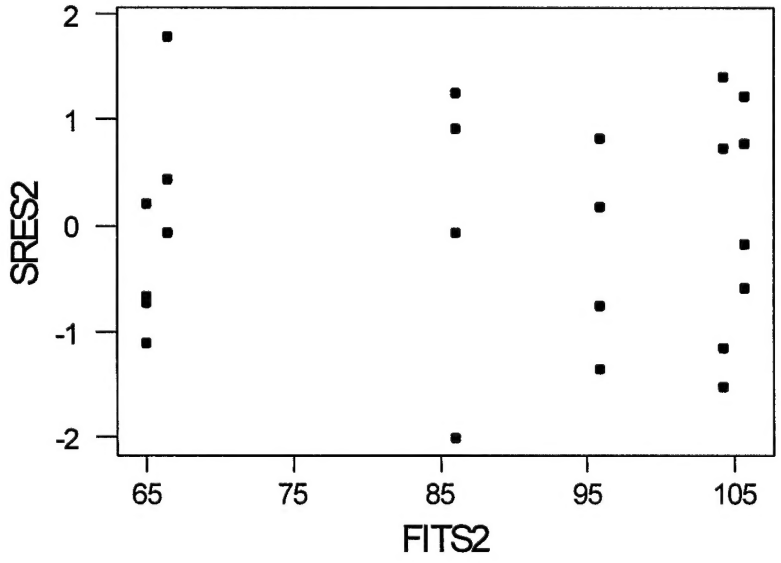


Figure 19 -- Test for homogeneity of variance for loading level 107 using standardized residuals versus fitted values of one-way ANOVA

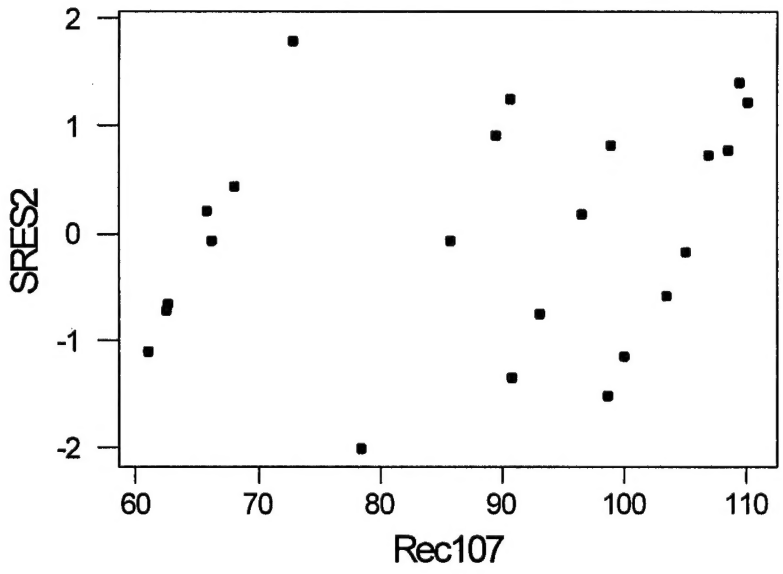


Figure 20 -- Test for linearity for loading level 107 using standardized residuals versus recovery rate of one-way ANOVA